A STUDY ON THE LEVEL OF SERUM NEOPTERIN A NEW NOVEL BIOMARKER IN ACUTE MYOCARDIAL INFARCTION

DISSERTATION

M.D. (BRANCH XIII)

BIOCHEMISTRY

GOVT. THANJAVUR MEDICAL COLLEGE, THANJAVUR
THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,
CHENNAI – INDIA.

APRIL 2011

BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON THE LEVEL OF SERUM NEOPTERIN A NEW NOVEL BIOMARKER IN ACUTE MYOCARDIAL INFARCTION"** is a bonafide record of the work done by **Dr. E. SRI VATSAN,** under our guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur, during the period of his Postgraduate study for M.D. (Branch XIII) Biochemistry from 2008 to 2011.

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ABBREVIATIONS

AT	-	Absorbance Test
AS	-	Absorbance Sample
ACS	-	Acute Coronary Syndrome
AMI	-	Acute Myocardial Infarction
BP	-	Blood Pressure
BH4	-	Tetrahydrobiopterin
C	-	Centigrade
CAD	-	Coronary Artery Disease
CK-MB	-	Creatine Kinase – MB Iso form
CM	-	Centimetre
CRP	-	C-reactive Protein
CVS	-	Cardio Vascular System
df	-	Degree of freedom
dl	-	deci liter
ECG	-	Electro Cardio Gram
FBG	-	Fasting Blood Glucose
G	-	Grams
GOD-POD	-	Glucose Oxidase – Peroxidase
GTP	-	Guonosine tri phosphate
GTP-CH	-	Guonosine tri phosphate – Cyclo hydrolase
HDL	-	High Density Lipo proteins
HIV	-	Human Immuno Deficiency Virus.
ICCU	-	Intensive Coronary Cardiac Unit
IHD	-	Ischaemic Heart Disease
L	-	Litre
LDL	-	Low Density Lipo proteins

LVEF	-	Left Ventricular Ejection Fraction
MACE	-	Massive Adverse Cardiac Events
MMOL	-	Milli Mol / litre
ML	-	Milli litre
μL	-	Micro litre
N	-	Neopterin
NH_2 TP	-	Dihydro neopterintriphosphate
nm	-	nano meter
nmOL/l	-	Nano mole / litre
NQMI	-	Non Q Wave Myocardial Infarction
mg	-	Milligram
Min	-	Minutes
O.D	-	Optical Density
OP	-	Out Patient
P	-	Probability
PH	-	Previous Hospitalisation
RT	-	Room Temperature
Sig	-	Significance
Std	-	Standard
SR	-	Serum
T	-	Temperature
T-Cell	-	Thymus derived
TIMI	-	Thrombolysis in myocardial infarction
VLDL	-	Very Low Density Lipo proteins
U	-	Units
US	-	Unstable Angina

THIS WORK IS DEDICATED

TO THE ALMIGHTY

&

TO MY BELOVED TEACHERS

I KEEP SIX HONEST SERVING MEN;

THEY TAUGHT ME ALL I KNOW,

THEIR NAMES ARE

WHAT, WHY, WHEN, WHERE, WHO AND HOW?

ACKNOWLEDGEMENT

I express my sincere gratitude to **THE DEAN** and **THE MEDICAL SUPERINTENDENT** and the **Professor and H.O.D of Cardiology** of our institution for permitting me to carry out the study.

My immense gratitude to my guide and my teacher **DR.N. SASIVADHANAM**, **Associate Professor and H.O.D, Department of Biochemistry, Thanjavur Medical College, Thanjavur,** for her constructive suggestions and constant encouragement throughout the period of my study.

I express my sincere gratitude to my co-guide **Dr. ELANGO**, **Associate Professor, Department of Biochemistry, Thanjavur Medical College, Thanjavur,** for his innovative suggestions and critical evaluation of the work.

I sincerely thank the **Assistant Professors**, Department of Biochemistry for their constant encouragement throughout the work.

I owe my thanks to **all my Colleagues** for their support during my study.

I thank all the **Technical Staff,** Department of Biochemistry, Thanjavur Medical College**,** Thanjavur for their technical assistance in the study.

I express my thanks to **DMLT students** who helped me for the sample collection, centrifugation and storage.

I owe my special thanks to **my family members** for their moral support in conducting the study. I thank all my **patients** who were part of the study for their co-operation throughout the study.

Above all my sincere thanks and prayers to the **DIVINE FORCE** which guides me throughout my life towards the best.

CONTENTS

INTRODUCTION

Acute myocardial infarction (AMI) is one of the most common diseases in hospitalised patients in industrialised countries. The early (30-day) mortality rate from AMI is ~30%, with more than half of these deaths occurring before the stricken individual reaches the hospital. Although the mortality rate after admission for AMI declined by ~30% over the past two decades, approximately 1 of every 25 patients who survives the initial hospitalisation dies in the first year after AMI . Mortality is approximately four fold higher in elderly patients (over age 75) compared with younger patients (1).

AMI is one of the important medical emergencies admitted in the hospitals. It is characterised by the presence of clinical features like sudden onset of severe chest pain with dysponea, palpitation and profuse sweating leading to cardiac arrythmias, cardiogenic shock, cardiac failure and death if not treated early (2).

Among the biomarkers for diagnosis and prognosis in AMI, the Neopterin, a pteridine derivative is considered to be one of the new novel bio-markers which is found to be elevated in patients with AMI.

Neopterin was first isolated from larvae of bee in 1963 (3). Eventually Neopterin was identified as the fluorescent component that was elevated in the urine of mice with Ehrlich ascites tumour (4). Now it is proved that this is the exclusive product of monocytes / macrophages that have been stimulated

by interferon gamma, a cytokine that is produced by activated T-lymphocytes and nature killer cells (5).

Biochemically Neopterin belongs to pteridines group. It is a catabolic product of guonosine-tri-phosphate [GTP]. Its molecular formula is $C_9H_{11}N_5O_4$. Its molecular mass is 253.215. Neopterin is synthesised from GTP via GTP cyclohydrolase(GTP-CH). The activity of GTP-CH can be greatly enhanced by interferon gamma (6).

7,8 dihydroneopterintriphosphate (NH_2TP) is on the biosynthetic pathway of 5,6,7,8 tetrahydrobiopterin (BH_4). BH_4 represents the electron donor in the amino acid metabolic pathways. Human monocytes / macrophages lack the enzyme 6-pyruvoyl-tetra-hydro biopterin synthase which converts NH_2TP to 6-pyruvoyl-tetra-hydro biopterin. Thus, in these cells NH_2TP accumulates and after hydrolysis by phosphatases is excreted as dihydroneopterin or Neopterin (7).

Neopterin reflects the of monocytes / macrophages activity stimulated by interferon gamma, a cytokine that is produced by the activated T-lymphocytes the nature killer cells (8). Estimation of Serum levels of Neopterin is a new novel biomarker in AMI and seems to be a prognostic marker for MACE in AMI.

AIMS & OBJECTIVES

To elucidate the role of Neopterin levels and its short term prognostic significance in AMI, the following objectives are formulated.

1. Estimation of levels of Serum Neopterin in AMI.

2. Significance of levels of Serum Neopterin during the treatment in the ICCU.

3. Correlation of levels of Serum Neopterin with the other cardiac risk factors.

4. The role of Neopterin for the prognostic stratification in AMI.

REVIEW OF LITERATURE

Studies have reported an association between Neopterin and the extent of atherosclerosis. Neopterin is elevated in patients with CAD and peripheral vascular disease (9).

The atheromatous plaque in AMI is characterised by presence of T-cells and a large amount of monocytes / macrophages in the shoulder region of the plaque. Activation of monocytes / macrophages produce pro-inflammatory cytokines giving rise to a rupture prone plaque. The activation of monocytes / macrophages may be due to the interferon produced by activated T-lymphocytes and is reflected by the high levels of Neopterin (10).

A central role in the pathogenesis of AMI is attributed to monocyte / macrophage activation. Neopterin, a pteridine derivative is synthesised by monocyte / macrophage upon stimulation with interferon. Neopterin levels are a useful tool to monitor monocyte / macrophage in AMI (11).

The Serum Neopterin concentration is associated with presence of angiographically demonstrated complex defects in patients with AMI and may represents a marker of coronary disease activity (12).

Neopterin was identified as fluorescent component. It is clear that Neopterin can be detected in most body fluids (13).

Inflammation plays a key role in the pathogenesis of atherosclerosis. Infiltration of neutrophils and monocytes / macrophages is detected in the vessel wall in patients with CAD. Macrophages activated by interferon gamma synthesize metalloproteinases and Neopterin, a pteridine derivative that has been used as a immunomarker. Neopterin as a marker of macrophage activation is significantly increased in patients with AMI, supporting the hypothesis of monocyte / macrophage activation in patients with AMI (14).

Atherosclerosis is a complex disease that involves lipoprotein influx and modification increased pro-oxidant stress and inflammatory angiogenic and fibro proliferative responses inter mingled with extra cellular matrix and smooth muscle cell proliferation resulting in the formation of atherosclerotic plaque (15).

The concept of response to injury, hypothesis considers atherosclerosis as a chronic inflammatory response of the arterial wall to the injured endothelium (16).

Inflammation plays important role in the pathogenesis of atherosclerosis. Atherosclerosis remains the major cause of death and pre mature disability in the developed countries.

Formation of fatty streak, the earliest phase of atherogenesis involves recruitment of leucocytes due to expression of leucocyte adhesion molecule on endothelial cells in turn triggered by primary pro–inflammatory cytokines.

Subsequent migration of inflammatory cells into the sub-endothelial space requires chemotaxis controlled by chemokines induced by the primary cytokines. Mononuclear cells within this initial infiltrate as well as intrinsic vascular cells subsequently release growth factors that stimulate proliferation of smooth muscle cells and hence the progression of plaques (17).

Inflammation is a major feature of atherothrombosis, and there is growing evidence of an association between systemic inflammation and the occurrence of stroke, peripheral artery disease, unstable angina, and IHD. Such an inflammatory component may be the final common result of a variety of infectious and non-infectious inflammatory stimuli and of the individual immunologic and inflammatory response (18).

Triggers of plaque rupture and novel risk factors for ACS are : Neopterin, Lselectin**,** Macrophage inflammatory peptide, Interleukin, Intra Cellular Adhesion, Vascular Cell Adhesion, Leucocyte Count, Heat Shock Proteins, Matrix Metaloprotease 9 (MMMP 9) (19).

Inflammation plays a role in the pathogenesis IHD. It is now accepted that inflammatory processes takes place within the atheromartous lesions and significantly contribute to the progression of stenosis and acute coronary

events. Studies have shown an association between markers of inflammation and the presence of coronary atherosclerosis, as well as the exacerbation of inflammation during acute myocardial ischemia. Several inflammatory markers have been shown to represent true cardio vascular risk factors. In this respect the CRP, the Neopterin and the Leucocyte count are all of particular interest. Studies suggest that high levels of Serum Neopterin may be a marker of atheromatous plaque vulnerability and identify patients at increased risk of serious coronary events. The results of these studies reveal that Neopterin may be a marker of coronary disease activity rather than a marker of presence of CAD (20).

Inflammation plays a vital role in atherosclerosis and coronary heart disease. Inflammatory processes of the coronary arterial wall are involved in plaque formation, progression and finally plaque instability leading to clinical manifestations of stable coronary artery disease and acute coronary syndromes. Biomarkers of inflammation emerged as potentially useful tools for risk stratification (21).

Studies also found an association between Neopterin levels and impaired LVEF (22).

Elevated Serum levels of Neopterin, an immune modulator secreted by activated macrophages with patients in ACS. Serum Neopterin is an

independent predictor of MACE in patients with ACS. This marker of macrophage may be useful for risk stratification in patients with AMI (23).

Alteration in Serum Neopterin correlate with macrophage activation and TIMI risk course. Serum Neopterin concentrations have a high correlation with TIMI risk course and may represent a marker useful in stratify patients with ACS (24).

Clinical studies performed recently proved great role of inflammatory processes in development of atherosclerosis. These inflammatory biomarkers are used in the diagnosis and identification of patients with unstable angina pectoris and myocardial infarct. (25).

Serum Neopterin concentrations have a high correlation with TIMI risk scores and may represent a marker useful in stratifying patients with ACS (26).

Patients with AMI and UA have significantly have higher levels of Neopterin than patients with remote AMI or Control group patients (27).

Neopterin was significantly increased in patients with stable CAD and more pronounced in patients with AMI than Control group patients (28).

Neopterin is significantly increased in patients with ACS and elevated Neopterin levels predict MACE during follow-up (29).

Rapid CAD progression in patients with stable CAD is associated with increased Neopterin levels (30).

Serum Neopterin levels is an independent predictor of MACE (31).

Patients who developed MACE during follow-up had significantly higher Neopterin levels than control group patients (32).

Patients with stable CAD and with MACE during follow-up had significantly higher Neopterin levels than those without MACE (33).

High Serum Neopterin levels are associated with the presence of demonstrated complex lesions (34).

Neopterin levels are a useful marker of the severity CAD (35).

Serum Neopterin levels predict MACE in patients with AMI (36).

Serum neopterin levels predict MACE in patients with NQMI (37).

Neopterin levels are increased after stroke (38).

Patients with carotid athersclerosis have higher Neopterin levels than in patients without (39).

Neopterin a by-product of guonosine tri-phosphate pathway, is produced by activated macrophages and serves as an activation marker for monocytes/ macrophages (40).

Long term prognostic value of Neopterin a novel marker of monocyte activation in patients with acute coronary sydrome. Neopterin is a soluble marker of monocyte activation and elevated levels are of prognostic values (41).

Inflammation plays a key role in the initiation and progression of atherosclerosis but also in the patho-physiology of atheromatous plaque disruption and the development of ACS.

Neopterin is a marker of inflammation and of immune system activation, it is synthesised by macrophages that, once activated (42).

Increased monocyte activation detected by an elevated plasma Neopterin level identifies patients at long term risk of death or recurrent acute coronary events after ACS.

Monocyte activation is believed to be playing an important role in the pathogenesis of ACS. Neopterin is a soluble marker of monocyte activation, and elevated levels are of prognostic value in patients with ACS (43).

Neopterin levels predict future major cardiac and vascular adverse events. This molecule can be considered as a useful marker of atherosclerotic plaque activity, permitting the identification of the subjects at highest risk for Major Adverse Cardiovascular Events. In line with above mentioned evidences, patients with high Neopterin levels may

require aggressive risk factor modification and intensive medical treatment irrespective of the severity of their CAD. This data suggest a potential clinical use of Neopterin as a marker for disease activity in patients with Cardiovascular Disease (44).

Recent data indicate that Serum levels of Neopterin are elevated in patients with coronary artery disease (CAD) and seem to be a prognostic marker for Major Adverse Cardiovascular Events. Based on many recent research works all over the world this molecule can be rendered a useful inflammatory marker of atherosclerotic plaque activity, permitting the identification of subjects at higher risk for Major Adverse Cardiovascular Events (45).

Neopterin levels a marker of macrophage activation predict cardiovascular events in ACS (46).

Neopterin is an independent predictor of all causes and cardio vascular mortality in individuals with or without CAD (47).

Serum Neopterin levels can be considered as an independent predictor of major adverse cardiac events. This marker of macrophage activation may be useful for risk stratification in cardiac patients (48).

Neopterin concentrations usually correlate with the activity of the disease. Elevated Neopterin concentration are among the best predictors of

adverse outcome in patients in cardiovascular diseases, HIV infections and various types of cancers (49).

Neopterin is an activation marker for monocytes / macrophages, and circulating levels of Neopterin are elevated in patients with coronary complex lesions in AMI (50).

Neopterin is one the markers of inflammation produced by the macrophages when stimulated by interferons and released by activated T-cells (51).

BRAUNWALD had initially proposed risk stratification based on the history, physical examination and ECG. The American college of Cardiology / American Heart Association Guidelines Committee later refined and incorporated cardiac markers for risk stratification (52).

MATERIALS AND METHODS

Neopterin is an activation marker for monocytes / macrophages and its circulating levels in the Serum is considered to be one of the prognostic markers in the treatment of AMI.

INCLUSION CRITERIA

(1) All cases admitted in the ICCU with the clinical features of AMI and with the positive documentation like increased cardiac enzyme levels, ECG findings, ECHO findings, etc.

(2) The group of patients in the age group between 45 – 65 years were selected irrespective of the gender difference.

(3) The associated findings like Hypertension, Diabetes Mellitus and Hypercholesterolemia were also included in the study.

(4) Patients with acute symptoms only were selected irrespective of time of admission.

EXCLUSION CRITERIA

1. Patients with features with Koch's lesion.
2. Patients who attended OP with features of atypical chest pain.
3. Patients with HIV infection.
4. Patients who underwent renal transplant recently.
5. Patients with the clinical features of viral infection.
6. Patients with malignant diseases.
7. Patients with autoimmune diseases.
8. Patients who had recent blood transfusion.

The patients who had fulfilled the above criteria were selected for the study. The present study is the estimation of the Serum Neopterin in patients with AMI and its short term prognostic significance during hospitalisation. For this study the prior permission from the DEAN, Thanjavur Medical College, Thanjavur, the Professor and H.O.D, Department of Cardialogy, Thanjavur Medical College, Thanjavur and the Associate Professor and H.O.D, Department of Biochemistry was obtained.

To start with the study, a protocol was framed with inclusion, exclusion criteria, Aims and objectives, materials and methods and with the review of literatures and references taken from various international journals and from the prescribed Text books of Cardiology (Harrison's, Text Book of Medicine, Braunwald's heart disease).

For this study 40 patients were selected as study group and 40 patients were selected as control group, based on the inclusion / exclusion criteria. The study group were collected from the ICCU Department of Cardiology, Thanjavur Medical College, Thanjavur. The blood samples of the study group were properly stored in the deep freezer after perfect centrifugation. The blood samples of the control group were collected from the normal persons and after perfect centrifugation and they were stored in the deep freezer. During collection the usual precautions for venipuncture were observed. The grossly haemolytic, icteric and lipemic samples were discarded. Samples were centrifuged again before assay, to remove any particulate material.

The Quantitative estimation of Serum Neopterin was done using Neopterin ELISA, Enzyme linked Immuno sorbent Assay for the Invitro Diagnostic Quantitative Determination of Serum Neopterin, obtained from IBL International.

(I) QUANTITATIVE DETERMINATION OF SERUM NEOPTERIN

TEST PRINCIPLE: Solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) based on the basic principle of a competitive ELISA. An unknown amount of antigen in the sample and a fixed amount of enzyme labelled antigen compete for the antibody binding sites (rabbit-anti-neopterin). Both antigen-antibody complexes bind to the wells of the micro titre strips coated with a goat-anti-rabbit antibody. Unbound antigen is removed by washing. The intensity of the colour developed after the substrate incubation is inversely proportional to the amount of antigen in the sample.

MATERIALS SUPPLIED IN THE KIT

Quantity	Symbol	Component
1x 12x 8	MTP	Microtiter Plate
6x0.5ml	CAL A-F	Standard A-F
2x0.5ml	CONTROL 1+2	Control 1+2
1x0.1 ml	ENZCONJ-CONC	Enzyme Conjugate
1x5ml	ANTISERUM	Neopterin AntiSerum
1x17ml	TMB SUBS	TMB Substrate Solution
1x17ml	TMB STOP	TMB Stop Solution
1x50ml	WASH BUF CONC	Wash Buffer
1x18ml	ASSAY BUF	Assay Buffer
1x	FOIL	Adhesive Foil

PRE-TEST SETUP INSTRUCTIONS

(A) Preparation of Lyophilized or Concentrated Components

Dilute / Dissolve	Component	with	Diluents	Relation	Remarks	Storage	Stability
15ml	Wash buffer	285ml	Bidist. water	1:20		2-8^{0}c	1 month
25 microliter	Enzyme conjugate	5ml	Assay buffer	1:201	Store Protected from light	2-8^{0}c	24 hours

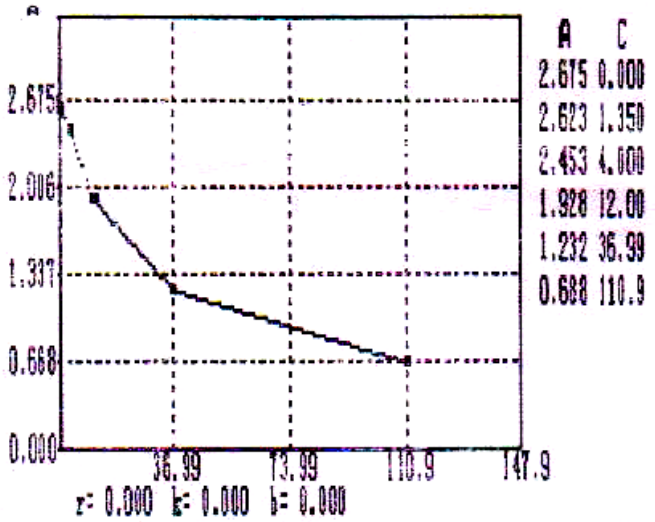
TEST PROCEDURE

MANUAL PROCEDURE

1	Pipette **10 µL** of each **Standard, Control, Serum sample and diluted urine sample** into the respective wells of the Microtiter Plate.
2	Pipette **100 µL** of freshly prepared **Enzyme Conjugate (1:201)** into each well.
3	Pipette **50 µL** of **Neopterin AntiSerum** into each well.
4	Cover plate with black adhesive foil**. Incubate 90 min** at **RT (18-25^{0}C)** on an orbital shaker (500 rpm) in the dark.
5	Remove adhesive foil. Discard incubation solution. Wash plate **4x** with diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
7	Pipette **150µL** of **TMB Substrate Solution** into each well.
8	**Incubate 10 min** at **RT(18-25^{0}C).**
9	Stop the substrate reaction by adding **150 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
10	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength600-650 nm) within **15 min.**

Normal Range of Serum Neopterin < 10 nmol / lit

QUALITY CONTROL FOR SERUM NEOPTERIN



(II) ESTIMATION OF CKMB

CKMB (Immuno inhibition / Modified IFCC Method)

Principle

CK- M fractions of the CK-MM and the CK-MB in the sample are completely inhibited by an anti CK-M antibody present in the reagent. Then the activity of the CK-B fraction is measured by the CK(NAC act) method. The CK-MB activity is obtained by multiplying the CK-B activity by two.

Reagents Preparation

Reagents are ready to use

Enzyme reagent (L1)

Starter reagent (L2)

Control : Reconstitute with 1ml of good quality D.W.Leave for 5 minutes to hydrate and mix by swirling to avoid frothing. The reconstituted control is stable for atleast 3 days when stored

Procedure

Wavelength / filter	:	340nm
Temperature	:	37^0 C /30^0 C/ 25^0 C
Light path	:	1 cm

Substrate Start Assay

Pipette into a clean dry test tube labelled Test (T)

Addition Sequence	(T) 25^0C / 30^0C/ 37^0C
Enzyme reagent (L1) Sample or Control (C)	0.8 ml 0.05 ml
Incubate at the assay temperature for 5 minutes and add	
Starter Reagent (L2)	0.2 ml

Mix well and read the initial absorbance A_0 after 5 minutes and repeat the absorbance reading after every 1, 2 & 3 minutes. Calculate the mean absorbance.

Change per minute (^A/ min).

Calculations

CK-MB activity in U/L 25°C / 30°C / 37°C = ^A / min. x 6666

REFERENCE RANGE

Serum CKMB levels up to 24 units/litre at 37^0C.

(III) ESTIMATION OF GLUCOSE

GLUCOSE OXIDASE PEROXIDASE (GOD/POD) METHOD– MANUAL AND AUTOANALYSER METHOD

Principle

Glucose is oxidised by glucose oxidase (GOD) to give gluconic acid and hydrogen peroxidase. The hydrogen peroxide formed is broken down by peroxidase to water and oxygen. The later oxidises phenol which combines with 4-aminophenazone to give a red coloured complex. The intensity of the red coloured complex is proportional to the concentration of glucose in the test. The intensity of the coloured complex is measured colorimetrically at 515 nm (500-530).

Reagents

1) Glucozyme Reagent I - Enzyme – chromogen tablets
2) Glucozyme Reagent II - Phenol solution
3) Glucozyme standard - 100mg / dl

PROCEDURE

Reagents	Blank (B) (mL)	Standard (S) (mL)	Test (T) (mL)
Glucozyme working reagent	1	1	1
Serum or plasma	-	-	0.01
Standard solution	-	0.01	-
Distilled water	0.01	-	-

Mix the contents of the test tubes thoroughly, and place at 37^0C for 15 minutes. Measure the optical density (OD) of the test and the standard against blank at 515 nm (Range 500-530). The final colour complex is stable for more than two hours at room temperature.

CALCULATION

Stock standard concentration = 1000mg%

Working Standard = 100mg%

100mL contains = 100mg%

Concentration of the Standard = 0.01mg%

ACTUAL VOLUME OF BLOOD

Actual Volume of Blood taken = 0.01ml

Glucose present in 100mL of plasma / Serum

$$= \frac{\text{O.D (T)} - \text{O.D (B)} \times \text{conc of std}}{\text{O.D (S)} - \text{O.D (B)} \times \text{vol of std}} \times 100$$

$$= \frac{T-B}{S-B} \times \frac{0.01}{0.01} \times 100$$

$$= \frac{T-B}{S-B} \times 100$$

Normal Value

Fasting Blood Glucose - 80 – 120 mgs/ dl.

(IV) ESTIMATION OF TOTAL CHOLESTEROL

The test is performed in the reagent kit by Enzymatic cholesterol esterase method.

PRINCIPLE: The free cholesterol liberated from the cholesterol esters by cholesterol esterase is oxidised by cholesterol oxidase to Cholesterol-4-en-3-one with the simultaneous production of hydrogen per oxide. The hydrogen per oxide reacts with 4-amino anti pyrine and a phenolic compound in the presence of peroxidase to yield a red coloured complex.

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholesterol-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4- Aminoantipyrene} \xrightarrow{\text{peroxidise}} \text{Red quinone} + 4H_2O.$$

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500nm.

REAGENTS

Reagent 1 (Enzymes/ Chromogen)

Cholesterol Esterase	≥ 30U/L
Cholesterol Oxidase	≥ 250U/L
Peroxidase	≥ 1000U/L
4-Aminoantipyrine	0.5mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.95	50mmol/L
Phenol	24mmol/L
Sodium Cholate	0.5mmol/L

Standard (Cholesterol 200 mg/dL)

Cholesterol	2g /L

Reagent Reconstitution

Allow the reagents to attain room temperature. Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A. Mix by gentle swirling.

Dispense into test tubes

	Blank	Standard	Test
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubate for 5 minutes at 37^0C. Mix and read at 500nm.

Normal value

<200 mgs/dl

(V) ACCUCARE

TRIGLYCERIDES-SLR

METHOD

Enzymatic colorimetric test

PRINCIPLE

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinonemine indicator is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of per oxidase.

REAGENTS

Reagent I	:	Enzyme reagent
Triglyceride Standard	:	200mg/dl

SAMPLE

Serum, heparinised plasma or EDTA plasma.

STABILITY

3 days at 2- 8^{0}C. 3 months at-20^{0}C.

REAGENT PREPARATION

Reagent is ready to use.

MANUAL PROCEDURE

Pipette into Test Tubes

	Blank	**Std**	**Sample**
Sample	_	_	10µl
Standard	_	10 µl	_
Reagent	1000 µl	1000 µl	1000 µl

Mix well, incubate for 5 minutes, at 37 ^{0} C (or 10 minutes at 20-25^{0} C). Measure absorbance of Sample (AT) and Standard (AS) against reagent blank at 505 nm. The colour is stable for 30 minutes at 20- 25^{0} C.

CALCULATION AND LINEARITY

AT /AS x conc. Std = mg / dl Triglycerides

This method is linear upto concentration of 1300 mg/dl. Dilute samples above this concentration 1 : 1 with 0.9% saline and reassay. Multiply the result by two.

REFERENCE VALUES

Men : 60 - 165 mg / dl

Women : 40 - 140 mg / dl

(VI) ESTIMATION OF HDL - CHOLESTEROL
PHOSPHOTUNGSTATE METHOD

PRINCIPLE

Chylomicrons, VLDL (Very Low Density Lipoproteins) and LDL Fractions in Serum or plasma are separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using Cholesterol esterase, Cholesterol Oxidase, Peroxidase and chromogen 4-Aminoantipyrine / Phenol.

REAGENTS

Reagent 1 (Enzymes / Chromogens)

Cholesterol Esterase	≥ 30 U /L
Cholesterol Oxidase	≥250 U /L
Peroxidase	≥ 1000U /L
4- Aminoantipyrine	0.5 mmol / L

Reagent 1 A (Buffer)

Pipes buffer, pH 6.95	50 mmol/ L
Phenol	24 mmol / L
Sodium Cholate	0.5 mmol / L

Reagent 2 (Precipitating Reagent)

Phosphotungstic Acid	2.4 mmol / L
Magnesium Chloride	39 mmol / L

Standard (HDL Cholesterol 50 mg / dL)

Cholesterol	0.5 g / L

REAGENT RECONSTITUTION

Allow the reagents to attain room temperature. Dissolve the contents of one bottle of reagent 1 into one bottle of reagent 1A. Mix by gentle swirling till completely dissolved. Write the Reconstitution Date in the space provided on the label of bottle 1 A. Wait for 5 minutes before using.

PROCEDURE

The samples, the precipitating reagent 2 and the reconstituted reagent should be brought to room temperature prior to use.

1. PRECIPITATION

Dispense into Centrifuge Tube

	Test
Sample	0.20 mL (200µ L)
Precipitating Reagent 2	0.20 mL (200µ L)

Mix well.

Centrifuge at 1500 g or 3500 -4000rpm for 10 minutes. Separate the clear supernatant immediately and determine the cholesterol content as given below.

HDL Cholesterol standard is not subjected to the precipitation step.

Dispense into test tubes.

	Blank	**Standard**	**Test**
Reconstituted Reagent	1ml	1ml	1ml
Standard	-	20µl	-
Supernatant	-	-	20µl

Incubate for 5 minutes at 37^0C. Mix and read.

Reference Value: 30- 70 mg /dl.

CALCULATED PARAMETERS

(VII) Freidwald's Formula :

Very Low Density Lipoprotein: = $\frac{\text{tryacylglycerol}}{5}$

(VIII) Low Density Lipoprotein = Total cholesterol – [HDL+VLDL]

RESULTS & DISCUSSION

Age Matched Statistical Analysis between the study group and the control group age less than 45 years

Table No. 1. T-Test

Sl. No	Group	Mean	Std. Deviation	Statistical inference
1	**BP1**			T = .352
	Test (n=3)	136.67	30.551	P > 0.05
	Control (n=4)	130.00	20.000	Not Significant
2	**BP2**			T = .849
	Test (n=3)	90.67	4.163	P > 0.05
	Control (n=4)	85.50	9.713	Not Significant
3	**FBG**			T = 1.081
	Test (n=3)	119.33	37.541	P > 0.05
	Control (n=4)	99.50	4.726	Not Significant
4	**CK_MB**			T = 5.300
	Test (n=3)	45.33	10.066	P < 0.05
	Control (n=4)	16.50	4.123	**Significant**
5	**N**			T = 2.319
	Test (n=3)	22.57	13.568	P > 0.05
	Control (n=4)	7.21	1.635	Not Significant
6	**PH**			T = 2.390
	Test (n=3)	3.67	.577	P > 0.05
	Control (n=4)	3.00	.000	Not Significant
7	**MACE**			T = 2.390
	Test (n=3)	3.67	.577	P > 0.05
	Control (n=4)	3.00	.000	Not Significant
8	**D**			T = 2.390
	Test (n=3)	3.67	.577	P > 0.05
	Control (n=4)	3.00	.000	Not Significant
9	**SR.CHOLE**			T = 3.353
	Test (n=3)	193.33	17.898	P > 0.05
	Control (n=4)	153.50	13.772	Not Significant
10	**TGL**			T = -.374
	Test (n=3)	125.00	6.557	P > 0.05
	Control (n=4)	128.00	12.463	Not Significant
11	**HDL**			T = -1.036
	Test (n=3)	36.00	12.490	P > 0.05
	Control (n=4)	46.00	12.728	Not Significant
12	**LDL**			T = 3.166
	Test (n=3)	132.33	16.773	P > 0.05
	Control (n=4)	76.25	26.625	Not Significant
13	**VLDL**			T = -.319
	Test (n=3)	25.00	1.000	P > 0.05
	Control (n=4)	25.50	2.517	Not Significant

Df = 5

Age Matched Statistical Analysis between the study group and the control group age less than 45 years

From the data illustrated in the Table No. 1 age matched statistical analysis between the study group and the control group of age less than 45 years is analysed. The following results are obtained as the statistical outcome. The parameters are analysed between the study and the control group. Among the parameters, there is a significant difference in the levels of CKMB (P Value < 0.05) which is statistically significant.

The other para meters between the study and the control group are not showing statistically significant difference.

Age Matched Statistical Analysis between the study group and the control group age between 45 to 55 years

Table No. 2.
T-Test

Sl. no	Group	Mean	S.D	Statistical inference
1	**BP1**			T = .822
	Test (n=13)	130.31	17.997	P > 0.05
	Control (n=18)	125.44	14.901	Not Significant
2	**BP2**			T = .952
	Test (n=13)	85.85	9.881	P > 0.05
	Control (n=18)	83.11	6.106	Not Significant
3	**FBG**			T = .451
	Test (n=13)	113.38	27.488	P > 0.05
	Control (n=18)	109.11	24.916	Not Significant
4	**CK_MB**			T = 5.445
	Test (n=13)	47.15	21.575	P < 0.05
	Control (n=18)	18.67	4.887	**Significant**
5	**N**			T = 4.726
	Test (n=13)	37.55	28.227	P < 0.05
	Control (n=18)	6.28	1.160	**Significant**
6	**PH**			T = 5.191
	Test (n=13)	3.62	.506	P < 0.05
	Control (n=18)	3.00	.000	**Significant**
7	**MACE**			T = 6.155
	Test (n=13)	3.69	.480	P < 0.05
	Control (n=18)	3.00	.000	**Significant**
8	**D**			T = 3.799
	Test (n=13)	3.46	.519	P < 0.05
	Control (n=18)	3.00	.000	**Significant**
9	**Sr.chole**			T = 5.028
	Test (n=13)	213.08	32.607	P < 0.05
	Control (n=18)	166.83	18.402	**Significant**
10	**TGL**			T = -1.087
	Test (n=13)	121.00	12.281	P > 0.05
	Control (n=18)	128.22	21.493	Not Significant
11	**HDL**			T = -4.881
	Test (n=13)	34.31	7.005	P < 0.05
	Control (n=18)	47.89	8.065	**Significant**
12	**LDL**			T = 6.073
	Test (n=13)	154.46	34.452	P < 0.05
	Control (n=18)	93.50	21.443	**Significant**
13	**VLDL**			T = -.927
	Test (n=13)	24.15	2.609	P > 0.05
	Control (n=18)	25.44	4.488	Not Significant

Df = 29

Age Matched Statistical Analysis between the study group and the control group age between 45 - 55 years

From the Table No. 2. the data illustrated is the statistical analysis between the study group and the control group. In the age group of between 45 to 55 years. It is clear that the Serum levels of CKMB, Neopterin, Cholesterol, HDL, LDL are having statistically significant difference between the 2 groups at the P level $P<0.05$ significant.

The parameters like systolic Blood Pressure, diastolic blood pressure, FBG are not having significant difference between the two groups.

P Value more than 0.05 not significant.

The parameters like History of previous hospitalisation, MACE and death are having significant difference between the two groups

P Value less than 0.05 significant.

Age Matched Statistical Analysis between the study group and the control group age more than 55 years

Table No. 3.

T-Test

Sl. No	Group	Mean	S.D	Statistical inference
1	**BP1**			T = .211
	Test (n=24)	127.00	22.341	P > 0.05
	Control (n=18)	125.78	11.680	Not Significant
2	**BP2**			T = .000
	Test (n=24)	83.67	9.898	P > 0.05
	Control (n=18)	83.67	7.236	Not Significant
3	**FBG**			T = .116
	Test (n=24)	106.42	19.675	P > 0.05
	Control (n=18)	105.61	25.435	Not Significant
4	**CK_MB**			T = 2.405
	Test (n=24)	41.33	21.902	P > 0.05
	Control (n=18)	26.28	17.306	Not Significant
5	**N**			T = 5.846
	Test (n=24)	25.68	13.413	P < 0.05
	Control (n=18)	7.05	1.551	**Significant**
6	**PH**			T = 2.124
	Test (n=24)	3.21	.415	P > 0.05
	Control (n=18)	3.00	.000	Not Significant
7	**MACE**			T = 4.140
	Test (n=24)	3.50	.511	P < 0.05
	Control (n=18)	3.00	.000	**Significant**
8	**D**			T = 2.124
	Test (n=24)	3.21	.415	P > 0.05
	Control (n=18)	3.00	.000	Not Significant
9	**SR.CHOLE**			T = 4.891
	Test (n=24)	213.63	40.858	P < 0.05
	Control (n=18)	163.39	17.161	**Significant**
10	**TGL**			T = .659
	Test (n=24)	135.08	18.606	P > 0.05
	Control (n=18)	130.28	28.617	Not Significant
11	**HDL**			T = -3.026
	Test (n=24)	34.83	9.361	P < 0.05
	Control (n=18)	43.33	8.506	**Significant**
12	**LDL**			T = 6.198
	Test (n=24)	151.71	36.901	P < 0.05
	Control (n=18)	92.22	19.681	**Significant**
13	**VLDL**			T = .745
	Test (n=24)	27.08	3.647	P > 0.05
	Control (n=18)	26.00	5.760	Not Significant

Df=40

Age Matched Statistical Analysis between the study group and the control group age more than 55 years

From the Table 3. the data obtained is the age matched Statistical Analysis between the study group and the control group of age more than 55 years.

From the table is clear that there is statistically significant difference between the study and the control groups for the following parameters like Serum Neopterin, MACE, Serum cholesterol, HDL. LDL.

The P value Less than 0.05 significant

The statistical difference with the other parameters like systolic blood pressure, diastolic blood pressure, FBG, CKMB, pH, Death, TGL and VLDL is not significant.

P value more than 0.05.

Sex Matched Statistical Analysis between the study group and the control group
Females
Table No. 4.
T-Test

Sl. No	Group	Mean	S.D	Statistical inference
1	**BP1**			T = -.071
	Test (n=12)	120.33	20.535	P > 0.05
	Control (n=13)	120.77	7.981	Not Significant
2	**BP2**			T = -.034
	Test (n=12)	80.67	10.491	P > 0.05
	Control (n=13)	80.77	3.113	Not Significant
3	**FBG**			T = -.387
	Test (n=12)	105.67	20.214	P > 0.05
	Control (n=13)	109.54	28.637	Not Significant
4	**CK_MB**			T = 5.429
	Test (n=12)	52.75	21.730	P < 0.05
	Control (n=13)	19.08	5.220	**Significant**
5	**N**			T = 5.268
	Test (n=12)	21.57	10.015	P < 0.05
	Control (n=13)	6.80	1.427	**Significant**
6	**PH**			T = 1.547
	Test (n=12)	3.17	.389	P > 0.05
	Control (n=13)	3.00	.000	Not Significant
7	**MACE**			T = 2.445
	Test (n=12)	3.33	.492	P > 0.05
	Control (n=13)	3.00	.000	Not Significant
8	**D**			T = 1.547
	Test (n=12)	3.17	.389	P > 0.05
	Control (n=13)	3.00	.000	Not Significant
9	**SR.CHOLE**			T = 4.497
	Test (n=12)	222.83	44.315	P < 0.05
	Control (n=13)	164.00	15.722	**Significant**
10	**TGL**			T = 1.022
	Test (n=12)	136.42	21.177	P > 0.05
	Control (n=13)	125.62	30.426	Not Significant
11	**HDL**			T = -3.595
	Test (n=12)	33.08	10.689	P < 0.05
	Control (n=13)	46.23	7.429	**Significant**
12	**LDL**			T = 5.743
	Test (n=12)	162.33	40.708	P < 0.05
	Control (n=13)	89.38	20.271	**Significant**
13	**VLDL**			T = 1.011
	Test (n=12)	27.33	4.141	P > 0.05
	Control (n=13)	25.23	6.002	Not Significant

Df = 23

Sex Matched Statistical Analysis between the study group and the control group

Females

From the Table No. 4 Sex Matched Statistical Analysis between the study group and the control group in the Females is obtained.

It is clear from the data that the parameters like CKMB level, Serum Neopterin level, Serum Cholesterol, HDL and LDL are having statistically significant difference between the control and the study groups.

P Value Less than 0.05 significant.

The other parameters like systolic blood pressure, diastolic blood pressure, FBG, history of previous hospitalisation, Death, TGL and VLDL are not showing statistically significant difference.

P Value more than 0.05 not significant.

Sex Matched Statistical Analysis between the study group and the control group (Males)

Table No. 5.

T-Test

Sl. No.	Group	Mean	S.D	Statistical inference
1	**BP1**			T = .778
	Test (n=28)	132.43	20.780	P > 0.05
	Control (n=27)	128.59	15.270	Not Significant
2	**BP2**			T = .783
	Test (n=28)	86.71	8.798	P > 0.05
	Control (n=27)	84.96	7.733	Not Significant
3	**FBG**			T = .990
	Test (n=28)	111.36	24.897	P > 0.05
	Control (n=27)	105.15	21.431	Not Significant
4	**CK_MB**			T = 3.469
	Test (n=28)	39.57	19.687	P < 0.05
	Control (n=27)	23.22	14.833	**Significant**
5	**N**			T = 6.065
	Test (n=28)	32.62	22.172	P < 0.05
	Control (n=27)	6.68	1.436	**Significant**
6	**PH**			T = 4.749
	Test (n=28)	3.46	.508	P < 0.05
	Control (n=27)	3.00	.000	**Significant**
7	**MACE**			T = 7.411
	Test (n=28)	3.68	.476	P < 0.05
	Control (n=27)	3.00	.000	**Significant**
8	**D**			T = 4.103
	Test (n=28)	3.39	.497	P < 0.05
	Control (n=27)	3.00	.000	**Significant**
9	**SR.CHOLE**			T = 5.980
	Test (n=28)	207.25	32.929	P < 0.05
	Control (n=27)	163.93	18.562	**Significant**
10	**TGL**			T = -.816
	Test (n=28)	126.89	14.793	P > 0.05
	Control (n=27)	130.81	20.511	Not Significant
11	**HDL**			T = -4.249
	Test (n=28)	35.46	7.743	P < 0.05
	Control (n=27)	45.37	9.487	**Significant**
12	**LDL**			T = 7.371
	Test (n=28)	146.36	31.602	P < 0.05
	Control (n=27)	92.07	21.963	**Significant**
13	**VLDL**			T = -.530
	Test (n=28)	25.39	3.010	P > 0.05
	Control (n=27)	25.93	4.349	Not Significant

Df = 53

Sex Matched Statistical Analysis between the study group and the control group

Males

From the Table No. 5 the data obtained is Sex Matched Statistical Analysis between the study group and the control group in males.

From the data it is clear that the parameters like CKMB, Serum Neopterin, MACE, history of previous Hospitalisation, Death, Serum Cholesterol, HDL and LDL are having statistically significant difference between the control and test groups.

P Value less than 0.05 significant ($P < 0.05$)

The other parameters like systolic blood pressure, diastolic blood pressure, FBG, TGL, VLDL are not showing statistically significant difference between control and the test group.

P Value more than 0.05 not significant. ($P < 0.05$)

Independent Samples Test for Serum Neopterin Level between the control and the test group

Table No. 6.

Group Statistics					
	Group	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
N	Test	40	29.30	19.873	3.142
	Control	40	6.72	1.416	.224

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
N	Equal variances assumed	23.181	.000	7.170	78	.000	22.59	3.150	16.315	28.858
	Equal variances not assumed			7.170	39.396	.000	22.59	3.150	16.216	28.956

Independent Samples Test for Serum Neopterin Level between the control and the test group

From the table No. 6 the results obtained is the independent sample test between the study and the control group with respect to the levels of Serum Neopterin.

The results obtained show that there is statistically highly significant difference between the study and the control group with respect to the Serum Neopterin levels.

0.000 Less than 0.05 less than P sig. 2 tailed.

Highly Significant.

($P < 0.000 < 0.05$)

One Sample statistical analysis in the study group between Serum CKMB and Serum Neopterin Level

Table No. 7.

T-Test

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
Neopterin	40	1.95	.221	.035
CK_MB	40	1.78	.423	.067

One-Sample Test						
	Test Value = 25					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Neopterin	-660.475	39	.000	-23.05	-23.12	-22.98
CK_MB	-347.333	39	.000	-23.23	-23.36	-23.09

One Sample statistical analysis in the study group between Serum CKMB and Serum Neopterin Level

From the Table No. 7. One Sample statistical analysis in the study group between Serum CKMB and Serum Neopterin Level

The results obtained show that there is significant statistical difference is found in the study group between Serum Neopterin levels and the Serum CKMB levels.

0.000 less than 0.05 less than P Value

Highly Significant

($P < 0.000 < 0.05$)

CKMB – Highly Significant in the study group with respect to Serum Neopterin Level.

Chi-Square Test

Neopterin morethan 25 *MACE Cross tabulation
Study Group

Table No. 8.
Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
N25 * MACE	40	100.0%	0	.0%	40	100.0%

N25 * MACE Cross tabulation					
			MACE		Total
			Negative	Positive	
N25	Less 25	Count	17	1	18
		% within N25	94.4%	5.6%	100.0%
	More 25	Count		22	22
		% within N25		100.0%	100.0%
Total		Count	17	23	40
		% within N25	42.5%	57.5%	100.0%

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	36.135(b)	1	.000		
Continuity Correction(a)	32.374	1	.000		
Likelihood Ratio	46.824	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	35.232	1	.000		
N of Valid Cases	40				
a Computed only for a 2x2 table					
b 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.65.					

Chi-Square Test

Neopterin morethan 25 *MACE Cross tabulation
Study Group

From the Table No. 8. the data obtained is Chi-Square Test analysis between the Serum Neopterin levels more than 25 nmol/lit. and its association with MACE in the study group.

The statistical inference obtained in the study group proves that there is statistically highly significant association between the Serum Neopterin levels more than 25 nmol/lit and the occurrence of the MACE.

0.000 Less than 0.05 Less than P Value.

Highly Significant association between Serum Neopterin and MACE in the study group.

($P < 0.000 < 0.05$)

Chi-Square Test

Neopterin morethan 25 *Death Cross tabulation Study Group

Table No. 9.

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
N25 * D	40	100.0%	0	.0%	40	100.0%

N25 * D Crosstabulation					
			D		Total
			Negative	Positive	
N25	Less 25	Count	18		18
		% within N25	100.0%		100.0%
	More 25	Count	9	13	22
		% within N25	40.9%	59.1%	100.0%
Total		Count	27	13	40
		% within N25	67.5%	32.5%	100.0%

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	15.758(b)	1	.000		
Continuity Correction(a)	13.179	1	.000		
Likelihood Ratio	20.679	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	15.364	1	.000		
N of Valid Cases	40				
a Computed only for a 2x2 table					
b 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.85.					

Chi-Square Test

Neopterin morethan 25 *Death Cross tabulation
Study Group

From the Table No. 9 the result obtained is the Chi-Square Test analysis in the study group with respect to Serum Levels of Neopterin more than 25 nmol/lit and the occurrence of death in the study group.

The statistical inference is there is highly significant association between the Serum levels more than 25nmol/lit and the occurrence of death in the study group.

0.000 less than 0.05 less P value

Highly significant association between the high levels of Serum Neopterin and the occurrence of the Death.

($P < 0.000 < 0.05$)

Chi-Square Test
N10 *Blood Pressure Cross tabulation
Study Group

Table No. 10.

N1 * BP1G systolic

Sl. No	Nepterin	BP1G		Statistical inference
		Below 120 (n=23)	above 120 (n=17)	
1	Below10	1 (50%)	1 (50%)	X^2=.048 Df =1 P < 0.05 Significant
2	more than 10	22 (57.9%)	16 (42.1%)	

Diastolic

Sl. No	Nepterin	BP2G		Statistical inference
		Below 80 (n=19)	above 80 (n=21)	
1	Below10	1 (50%)	1 (50%	X^2=.005 Df =1 P < 0.05 Significant
2	more than 10	18 (47.4%)	20 (52.6%)	

Chi-Square Test
N10 *Blood Pressure Cross tabulation
Study Group

From Table No. 10 the data obtained is the Chi-square test analysis between the systolic blood pressure more than 120 mm of Hg. and its association with Serum Neopterin Levels more than 10nmol/lit.

The results show that there is significant association between the systolic blood pressure and the Serum Neopertin levels more than 10nmol / lit.

P Less than 0.05 significant.

From the same table the association between the diastolic blood pressure more than 80mm of Hg. and the Serum Neopertin levels more than 10nmol/lit is obtained and it is found that the association between the two is significant.

P Less than 0.05 significant.

There is significant association between the Serum Neopertin and the Hypertension.

($P < 0.000 < 0.05$)

SPEARMAN'S CORRELATIONS

Table No. 11.

	AGE	SEX	N	BP1	BP2	FBG	CK_MB	PH	MACE	D	SR.CHOLE	TGL	HDL	LDL	VLDL
AGE	1	.344(*)	-.178	-.165	-.234	-.058	-.146	-.406(**)	-.253	-.311	.217	.322(*)	.109	.185	.334(*)
SEX	.344(*)	1	-.279	-.325(*)	-.347(*)	-.092	.317(*)	-.282	-.320(*)	-.221	.158	.218	-.152	.248	.211
N	-.178	-.279	1	.315(*)	.363(*)	.224	-.312	.707(**)	.857(**)	.735(**)	-.270	-.132	-.081	-.286	-.117
BP1	-.165	-.325(*)	.315(*)	1	.878(**)	.079	-.254	.194	.230	.299	-.283	-.117	.036	-.359(*)	-.125
BP2	-.234	-.347(*)	.363(*)	.878(**)	1	.129	-.259	.324(*)	.234	.356(*)	-.351(*)	-.113	-.035	-.399(*)	-.116
FBG	-.058	-.092	.224	.079	.129	1	-.102	.166	.242	.332(*)	-.195	-.237	.058	-.162	-.235
CK_MB	-.146	.317(*)	-.312	-.254	-.259	-.102	1	-.056	-.127	-.222	.030	.038	.075	.012	.031
PH	-.406(**)	-.282	.707(**)	.194	.324(*)	.166	-.056	1	.666(**)	.565(**)	-.271	-.103	-.056	-.313(*)	-.109
MACE	-.253	-.320(*)	.857(**)	.230	.234	.242	-.127	.666(**)	1	.597(**)	-.287	-.138	-.031	-.313(*)	-.138
D	-.311	-.221	.735(**)	.299	.356(*)	.332(*)	-.222	.565(**)	.597(**)	1	-.433(**)	-.303	-.028	-.423(**)	-.279
SR.CHOLE	.217	.158	-.270	-.283	-.351(*)	-.195	.030	-.271	-.287	-.433(**)	1	.154	.254	.956(**)	.210
TGL	.322(*)	.218	-.132	-.117	-.113	-.237	.038	-.103	-.138	-.303	.154	1	-.331(*)	.106	.982(**)
HDL	.109	-.152	-.081	.036	-.035	.058	.075	-.056	-.031	-.028	.254	-.331(*)	1	.065	-.287
LDL	.185	.248	-.286	-.359(*)	-.399(*)	-.162	.012	-.313(*)	-.313(*)	-.423(**)	.956(**)	.106	.065	1	.158
VLDL	.334(*)	.211	-.117	-.125	-.116	-.235	.031	-.109	-.138	-.279	.210	.982(**)	-.287	.158	1
n	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).

SPEARMAN'S CORRELATIONS

From Table No. 11 the Spearman's Correlations are statistically analysed between the Serum Neopterin levels more than 10nmol/lit and the various parameters taken for the study.

The Serum Neopterin level more than 10 nmol/lit is having statistically highly significant relationship with the parameters like MACE, Death and the history of previous hospitalisation at the level 0.01 level (2 tailed) Highly Significant.

The Serum Neopterin level more than 10nmol/lit is having statistically significant relationship with blood pressure (both systolic and diastolic) at 0.05 level significant.

TABLE NO. 12.
FREQUENCY TABLE FOR AGE GROUP

Age Group		Frequency	Percent
Valid	Below 45 Years	3	7.5
	46 – 55 Years	13	32.5
	56 & Above	24	60.0
	Total	**40**	**100.0**

- 60% of patients are in the age group of more than 56 years.
- 32.5% of Patients are in the age group of 46 – 55 years.
- 7.5% of Patients are in the age group of below 45 years.

From the above data around 60% (ie) large group of AMI Patients are in the age group of more than 56 years. This data justifies that AMI occurrence is directly proportional to the increase in age group.

AMI α INCREASE AGE

This proves that as age advances the frequency of AMI occurrence is higher.

TABLE NO. 13.
FREQUENCY TABLE FOR SEX

Sex		Frequency	Percent
Valid	Male	28	70.0
	Female	12	30.0
	Total	**40**	**100.0**

- From the table 70% of Patients are found to be males.
- 30% of Patients are found to be female.

From the data it is evident that Males are having high risk and more prone for AMI then females.

It is justified that the occurrence of AMI is more common in males than the females.

Occurrence of AMI in Male > Occurrence of AMI in Female

TABLE NO. 14.
FREQUENCY TABLE FOR SYSTOLIC BLOOD PRESSURE

BP (Systolic)		Frequency	Percent
Valid	Below 120	23	57.5
	Above 120	17	42.5
	Total	**40**	**100.0**

- From the above Table 57.5% (23 patients) are having systolic Blood pressure below 120mm of Hg.
- 42.5% (17 Patients) are having systolic Blood Pressure more than 120.

According to the data the systolic blood pressure does not have any significant influence over AMI occurrence.

According to the previous studies there is no significant correlation between a systolic blood pressure and the occurrence of AMI.

The table justifies that there is no influence of the systolic blood pressure on the occurrence of AMI.

TABLE NO. 15.
FREQUENCY TABLE FOR DIASTOLIC BLOOD PRESSURE

BP (Diastole)		Frequency	Percent
Valid	Below 80	19	47.5
	Above 80	21	52.5
	Total	**40**	**100.0**

- From the above table it is evident 52.5% (21 patients) are having diastolic Blood Pressure above 80mm of Hg.
- 47.5% (19 Patients) are having diastolic Blood Pressure less than 80mm of Hg.

From Table it is evident that there is significant correlation between the diastolic Blood Pressure and the occurrence of AMI. More than 50% of patients are having diastolic Blood Pressure above normal.

From the table it is justified that the diastolic blood pressure is having influence on the occurrence of AMI.

TABLE NO. 16.
FREQUENCY TABLE FOR FBG

FBG		Frequency	Percent
Valid	Below 120	29	72.5
	Above 120	11	27.5
	Total	**40**	**100.0**

- From the table it is noted 72.5% (29 patients) are having fasting blood sugar below 120 and 27.5% (11 patients) are having fasting blood glucose more than 120.

From the table it is evident that the FBG does not influence the occurrence of AMI.

According to the data around more than 70% of patients who had AMI are showing FBG within normal limits and around 27% of patients with AMI are having abnormal FBG.

TABLE NO. 17.
FREQUENCY TABLE FOR CKMB

CKMB		Frequency	Percent
Valid	Less than 25	9	22.5
	More than 25	31	77.5
	Total	**40**	**100.0**

- From the table 77.5% (31 patients) are having CKMB levels more than 25, 22.5% (9 Patients) are having CKMB levels less than 25.

From the above table it is clear that the levels of CKMB are increased in around 77.5% of patients with AMI and around 22.5% of patients with AMI are having CKMB levels within normal limits.

This data justifies there is proportionate increase in the Serum level of CKMB in patients with AMI.

Serum levels of CKMB is having influence on the occurrence of AMI.

Serum levels of CKMB α AMI.

TABLE NO. 18.
FREQUENCY TABLE FOR NEOPTERIN

Neopterin		Frequency	Percent
Valid	Below 10	2	5.0
	Above 10	38	95.0
	Total	**40**	**100.0**

- From the above table 95% of patients (38 patients) are having Serum Neopterin level more than 10.
- 5% of patients (2 patients) are having Serum Neopterin level less than 10.

From the above table it is evident that Serum Neopterin level are more than normal in around 95% of patients with AMI and only 5% of patients is within normal limits.

This justifies that there is highly significant correlation between Serum Neopterin and the occurrence of AMI.

Serum Neopterin α AMI.

TABLE NO. 19.

FREQUENCY TABLE FOR PREVIOUS HOSPITALISATION

Previous Hospitalisation		Frequency	Percent
Valid	Negative	25	62.5
	Positive	15	37.5
	Total	**40**	**100.0**

- From the above table it is noted 62.5% (25 patients) are presented with previous history of hospitalisation.
- 37.5% (15 patients) are not presented with previous history of hospitalisation.

From the above table it is evident that there is no proportionate correlation between the occurrence of AMI and the previous history of hospitalisation.

TABLE NO. 20.
FREQUENCY TABLE FOR MACE

MACE		Frequency	Percent
Valid	Negative	17	42.5
	Positive	23	57.5
	Total	**40**	**100.0**

- From the above illustration 57.5% (23 patients) are having 'B' response (+)
- 42.5% (17 patients) are in the A response (–)

From the above table it is clear that the occurrences of MACE is associated in more than 50% of patients with AMI and around less than 50% of patients are not associated with MACE with AMI.

From this it is justified that the occurrence of the MACE has significant correlation in patients with AMI.

MACE α AMI

TABLE NO. 21.
FREQUENCY TABLE FOR DEATH

Death		Frequency	Percent
Valid	Negative	27	67.5
	Positive	13	32.5
	Total	**40**	**100.0**

- From the above table it is found 67.5% (27 Patients) are in the A response 32.5% (13 patients) are in the B response.

From the above table the mortality rate in patient with AMI is less than 50% that is around 32.5% of patients and the remaining 67.5% of patients are discharged without mortality. The mortality rate can further be reduced by early detection and intervention of MACE during hospitalisation.

TABLE NO. 22.
FREQUENCY TABLE FOR SERUM CHOLESTEROL

Serum Cholesterol		Frequency	Percent
Valid	Less than 200	15	38%
	More than 200	25	62%
	Total	**40**	**100.0**

From the table it is evident that more than 50% of patients with AMI are having Serum Cholesterol level more than 200 mg/dl. Therefore the high Serum cholesterol levels are having influence over the occurrence of AMI.

Increased Serum Cholesterol α occurrence of AMI

TABLE NO. 23.
FREQUENCY TABLE FOR SERUM HDL

HDL		Frequency	Percent
Valid	Less than 40	30	75%
	More than 40	10	25%
	Total	**40**	**100.0**

From the table it is evident that around 75% of patients with AMI are having Serum HDL levels less than 40 mg/dl and remaining 25% of patients with AMI are having HDL levels more than 40mg/dl.

Serum HDL 1/α occurrence of AMI.

Therefore from the table it is evident that low level of HDL is having influence over the occurrence of AMI. The high levels of Serum HDL will prevent the occurrence of AMI.

The present study was undertaken in patients who were admitted with clinical features of AMI in ICCU Thanjavur Medical College, Thanjavur. The study was started during November 2009 and was concluded during June 2010. The patients were in the age group of between 45 – 65 years including both genders with or without associated risk factors like Hypertension, Diabetes Mellitus, Hypercholesterolemia, etc. The levels of Serum Neopterin were estimated both in the study group and the control group and the levels were statistically correlated with other biochemical factors and clinical features.

Serum Neopterin levels were more than normal in the entire study group which signifies that Serum Neopterin levels were increased in AMI. The statistical correlation between the high levels of Neopterin and AMI was found to be highly significant [P(0.000) at 0.01 level 2 tailed]. In contrast Serum Neopterin level were found to be within normal limits in the control group which signifies that there is no disease activity in this group.

The high Serum Neopterin levels were associated with Massive Adverse Cardiac Events (MACE) like Cardiac Arrythmias, Cardiac Blocks, Cardiogenic Shock, Cardiac Failure, etc.

The correlation between the higher levels of Serum Neopterin more than 25 nmol/lit and the occurrence of MACE in the patients was statistically significant [P (0.000) at 0.01 level 2 tailed].

Hence, Neopterin can be considered not only as a biomarker for disease activity but also a prognostic marker to identify the high risk patients

during treatment and to implement emergency and essential medical intervention to prevent mortality. The study group with high Serum Neopterin levels were followed up critically during their hospitalisation and it is evident from the study that the occurrence of MACE and mortality is highly significant [P(0.000) at 0.01 level 2 tailed]. According to the above study it is proved that Serum levels of Neopterin can be considered as an independent biomarker for the disease activity and for the prognostic activity in AMI.

There is correlation between Serum Neopterin more than 25 nmol/lit and with previous history of hospitalisation and there is statistically significant correlation [P(0.000) at 0.01 level 2 tailed].

There is significant correlation with high levels of Serum Neopterin more than 25 and the occurrence of Death in patients with AMI. The statistical analysis shows that highly significant correlation [P(0.000) at 0.01 level 2 tailed] is found.

Apart from the above the Serum Neopterin levels were correlated with other biochemical markers like fasting blood glucose, lipid profile, CKMB levels and hypertension.

A significant correlation was found between the levels of Serum Neopterin and CKMB levels in the study group. And this correlation was found to be statistically significant [P(0.000) at 0.01 level 2 tailed].

Serum Neopterin levels were compared with fasting blood glucose, lipid profile and the correlation was found to be not significant statistically. The Serum Neopterin levels were correlated in the study group who had hypertension and there is significant correlation.

From these studies the major finding is that Neopterin, a biomarker for monocyte / macrophage activation and it is a prognostic marker to predict adverse cardiac events in patients suffering from AMI. When the levels of CKMB were added the disease activity was proved even stronger due to the statistically significant correlation that exists between higher Serum Neopterin levels and higher CKMB levels.

The studies reviewed, have demonstrated that Neopterin level is an important predictor of future cardiac as well as vascular adverse event. In particular, Neopterin levels predict future major cardiac and vascular adverse events in patients presenting with chronic coronary artery disease with acute coronary syndromes. In those with many of the above cited studies underline the strong association between high Neopterin levels and complex atherosclerotic lesions but not with disease extension giving account for the prognostic properties of Neopterin. This renders this molecule a useful marker of atherosclerotic plaque activity, permitting the identification of the subjects at highest risk for MACE.

Taken together, observations from all the studies reviewed propose the existence of a strong link between high Neopterin levels and cardiovascular risk profile, suggesting a potential clinical use of Neopterin as a marker for disease activity in subjects with cardiovascular disease. This could help in identifying patients who are at a higher risk of developing cardiovascular adverse events who might benefit from urgent preventive strategies exploitation or extensive diagnostic work-up, as well as actual therapy, depending from their co-morbidities.

SUMMARY

The major finding of this study is that Neopterin a marker for monocyte/ macrophage activation is predictive for adverse cardiac events in patients with AMI. This study has led to the understanding that inflammation, has reflected by the levels of Serum Neopterin, which is a major determinant of short term outcome in the study group. The results of this study show that Neopterin, a marker of macrophage activation, predicts adverse cardiovascular events during follow-up in patients with AMI.

By estimating the serum levels of Neopterin, detection of disease activity and intervention of MACE in AMI patients are possible and the mortality can be reduced in patients with AMI.

CONCLUSION

In this study it is proved that monocyte / macrophage activation in AMI is reflected by the levels of Neopterin and a strong correlation was found between high levels of Neopterin and MACE.

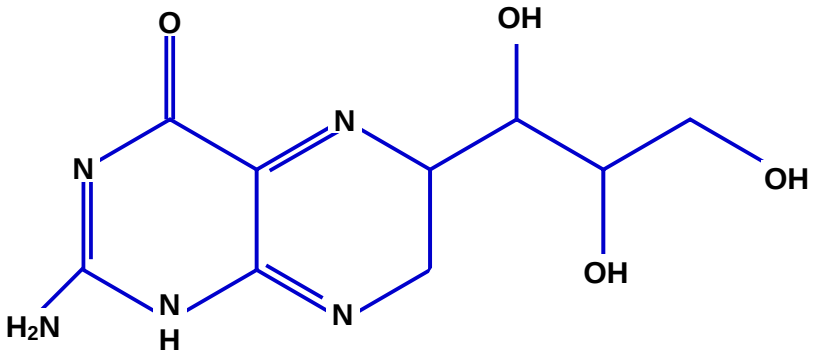
To conclude Neopterin concentrations are usually increased in AMI. Highly elevated Neopterin concentrations are among the best predictors of adverse outcome in patients with AMI risk.

Braunwald had initially proposed risk stratification based on the History, Physical examination and Electrocardiogram.

The American college of Cardiology / American Heart Association guide lines Committee later refined and incorporated cardiac markers for risk stratification.

This study postulates that Neopterin can be considered as a new novel Biomarker for the disease activity in AMI and also as a prognostic marker for risk stratification in AMI to prevent mortality and MACE.

NEOPTERIN



IUPAC Name	2-amino-6-(1, 2, 3-trihydroxypropyl)-1H-pteridin-4-one
Molecular formula	$C_9H_{11}N_5O_4$
Molar mass	253.215 g/mol

FIG. V

Role of Inflammation in the pathogenesis of AMI

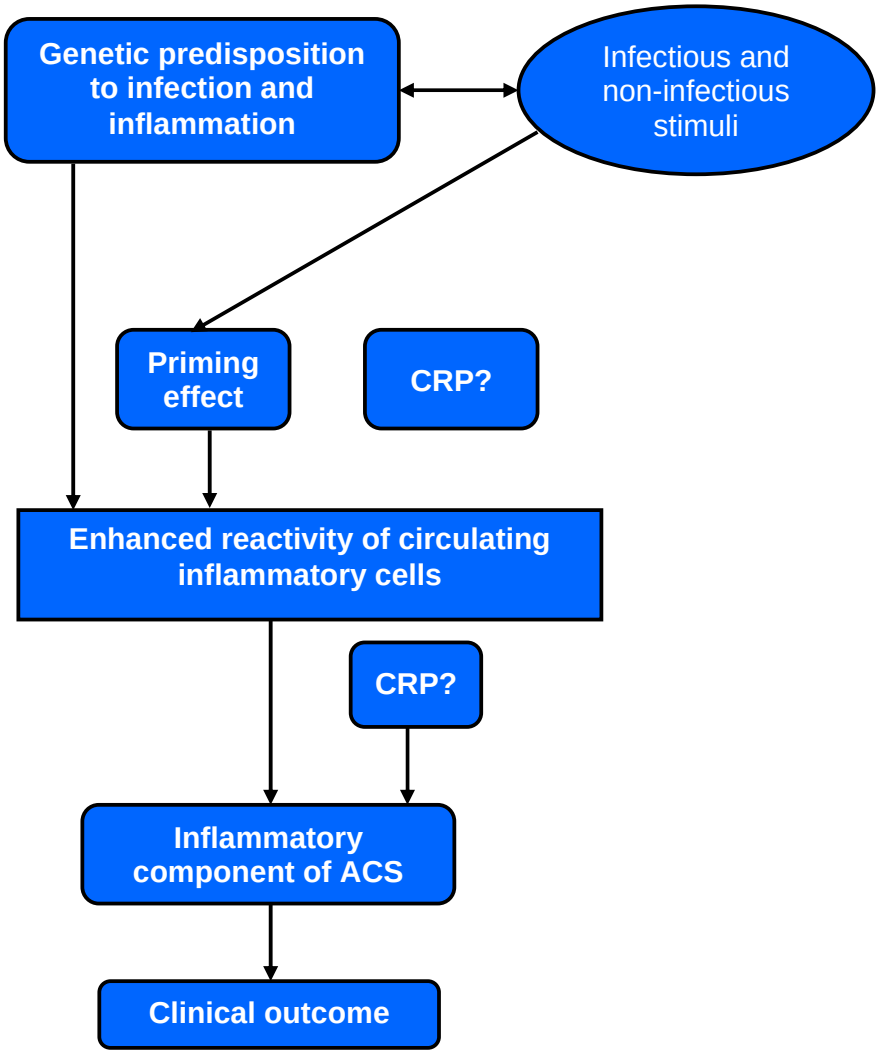


FIG. I

NEOPTERIN PRODUCTION BY THE MONOCYTE / MACROPHAGE

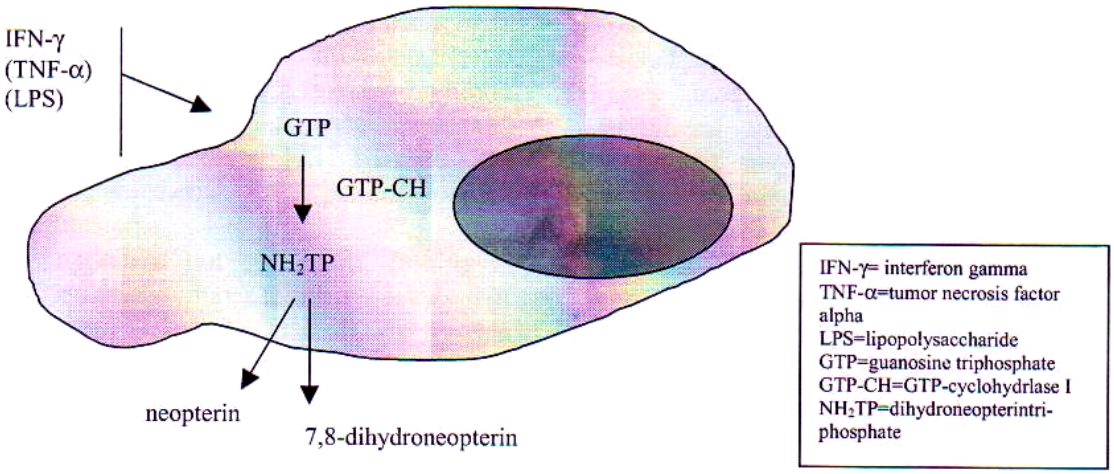


FIG. II
Pathogenesis of ACS

34

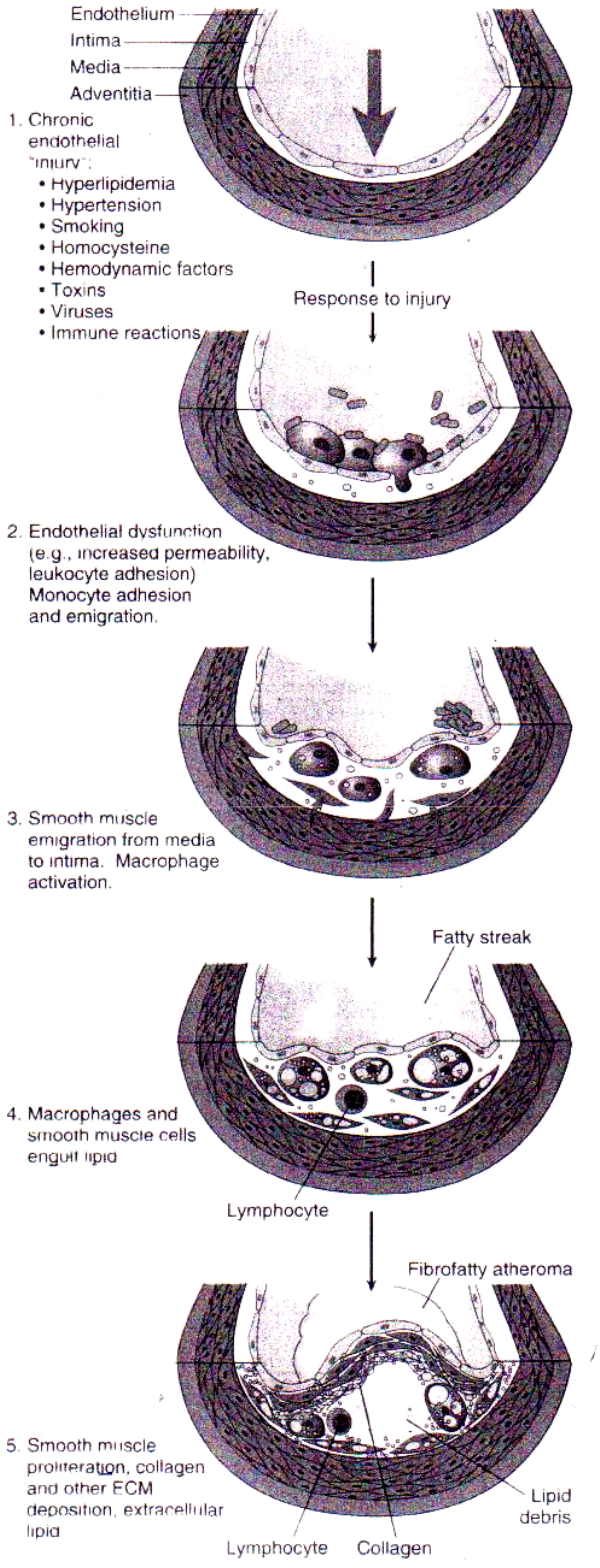


FIG. III

Pathogenesis of ACS

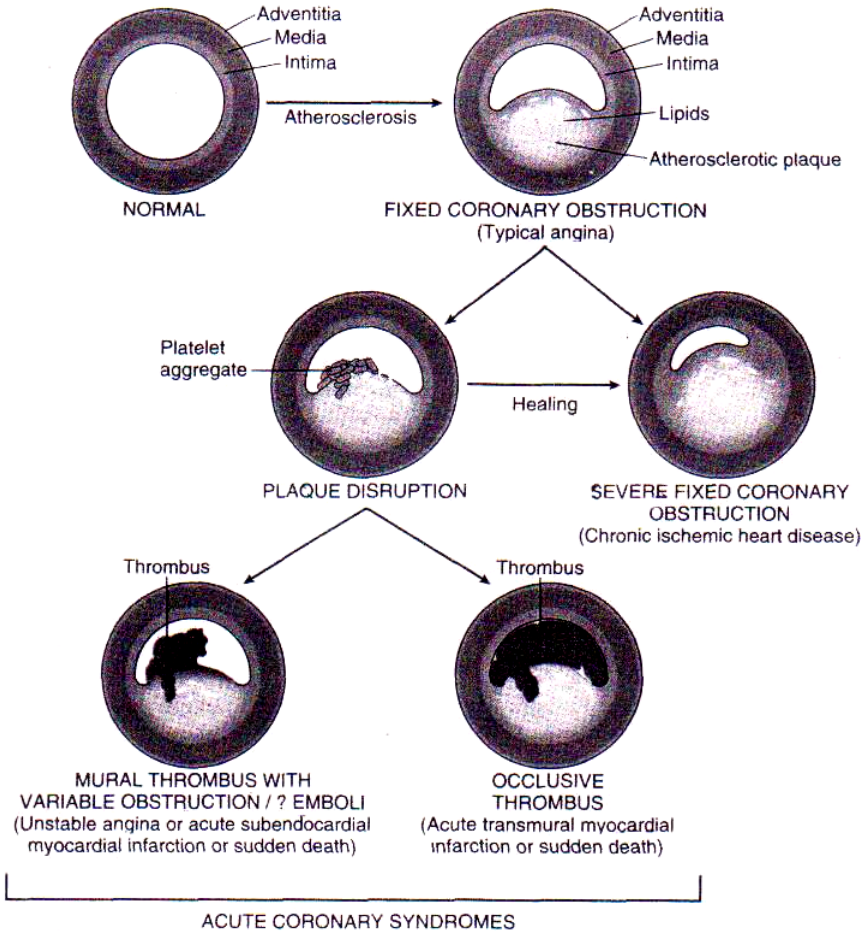


FIG. IV

Circulating levels of inflammatory markers provide a reflection of the underlying inflammatory response

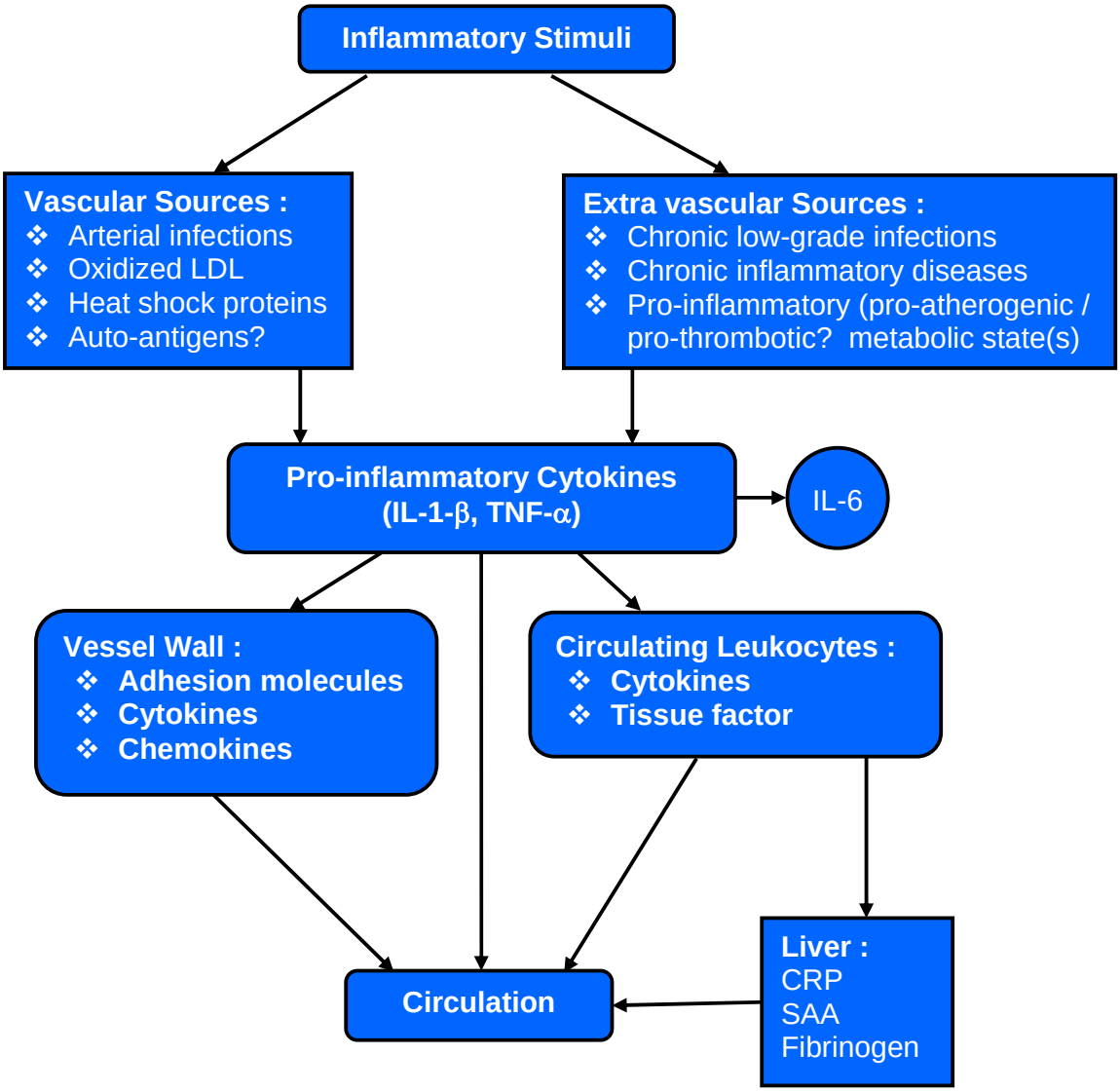


FIG. V

Role of Inflammation in the pathogenesis of AMI

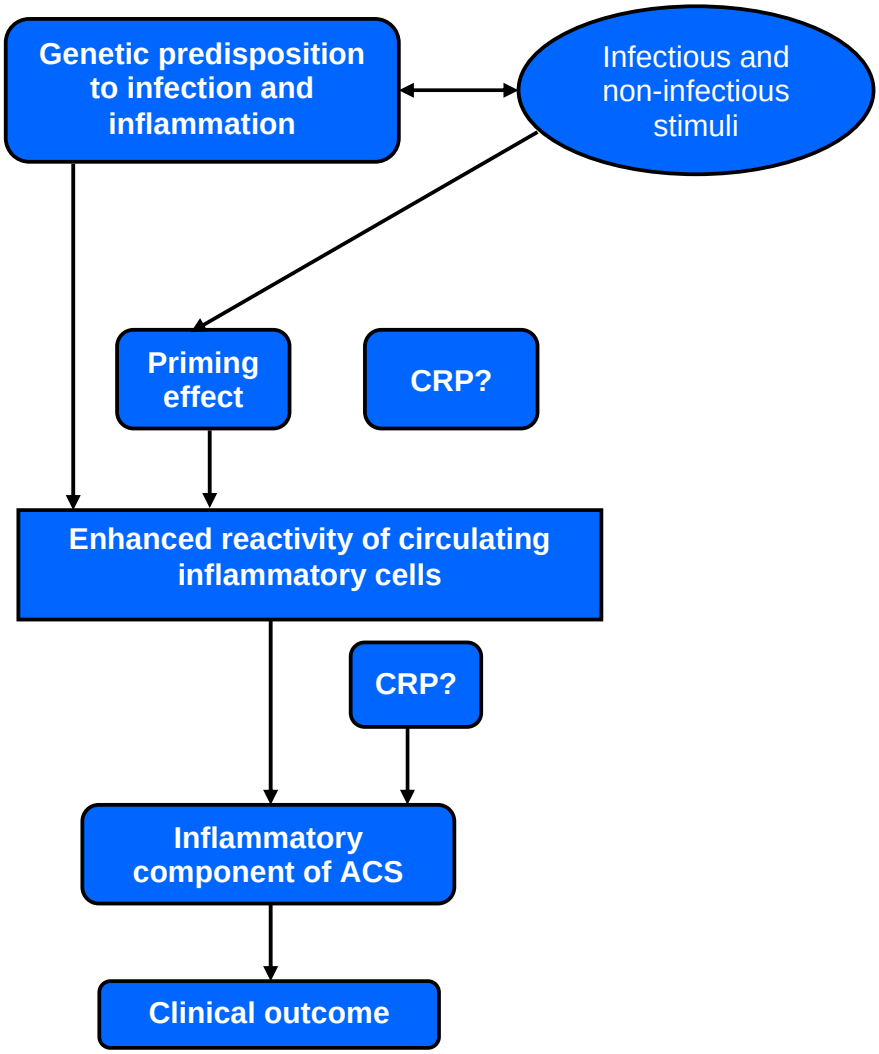


FIG – VI
FREQUENCY CHART FOR AGE GROUP

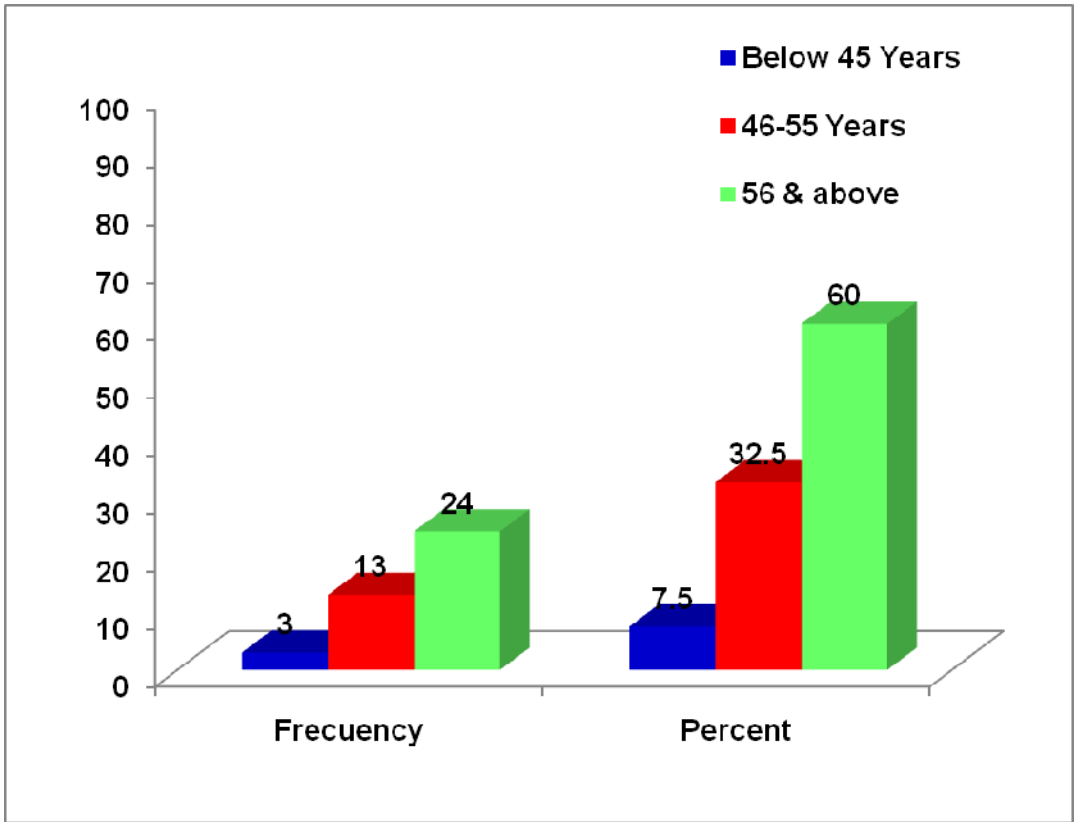


FIG – VII
FREQUENCY CHART FOR SEX

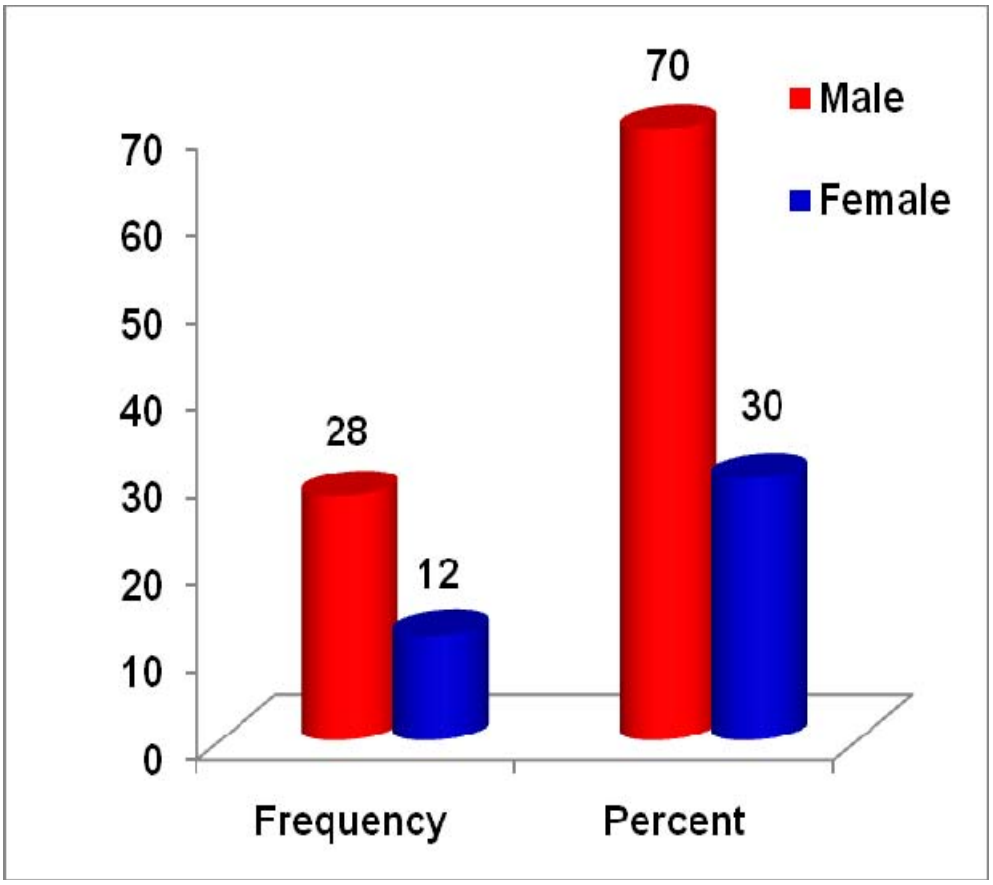


FIG – VIII

FREQUENCY CHART FOR SYSTOLIC BLOOD PRESSURE

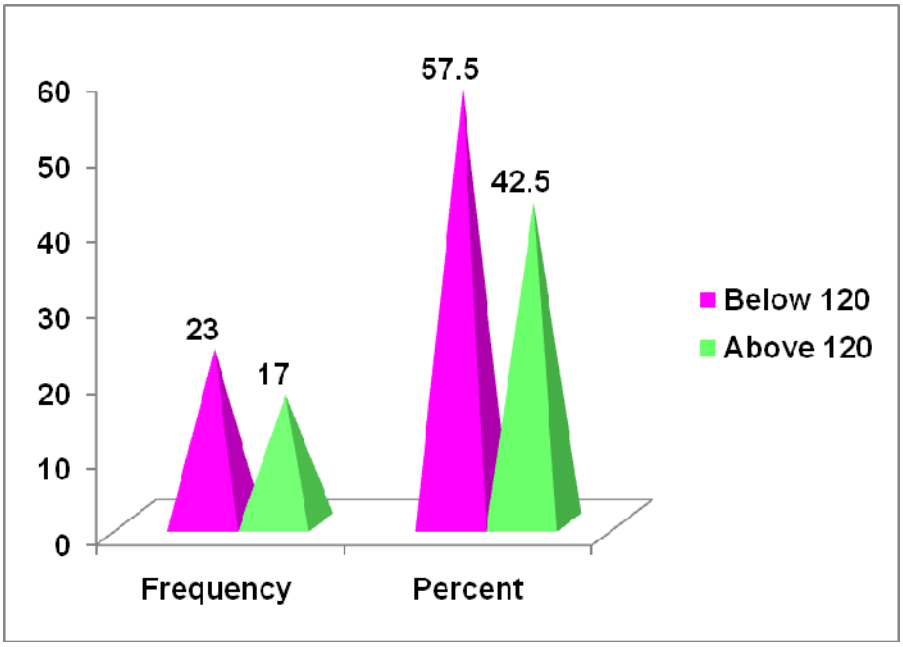


FIG – IX

FREQUENCY CHART FOR DIASTOLIC BLOOD PRESSURE

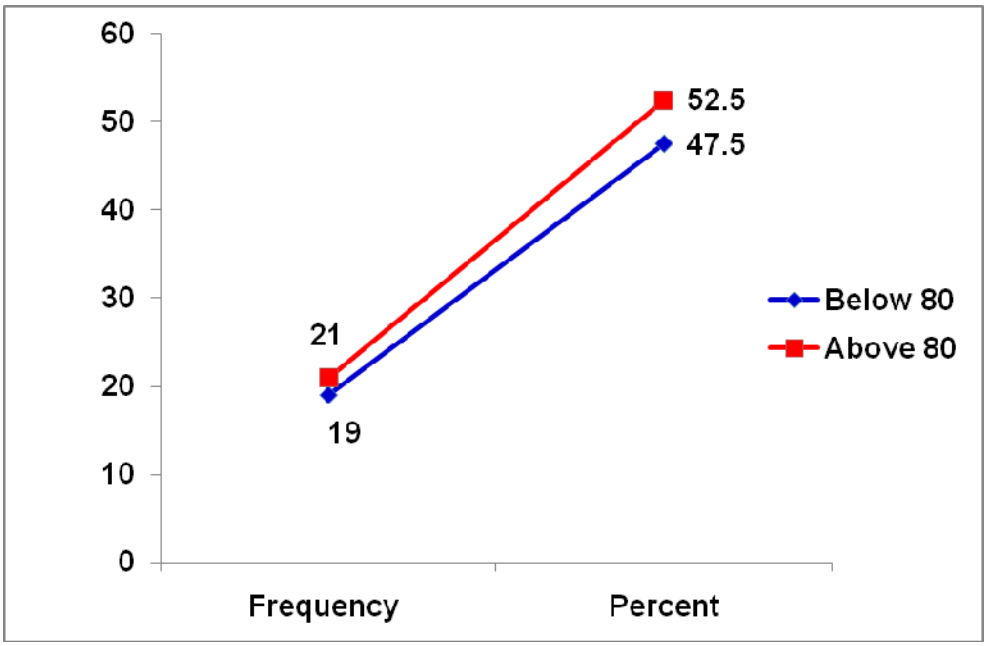


FIG – X
FREQUENCY CHART FOR FBG

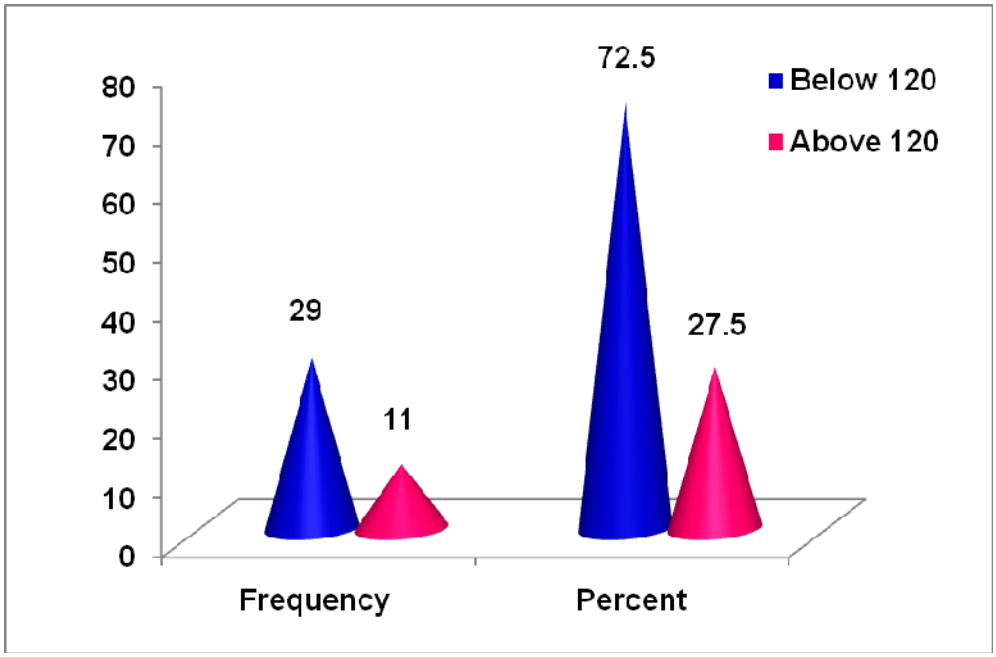


FIG – XI

FREQUENCY CHART FOR CKMB

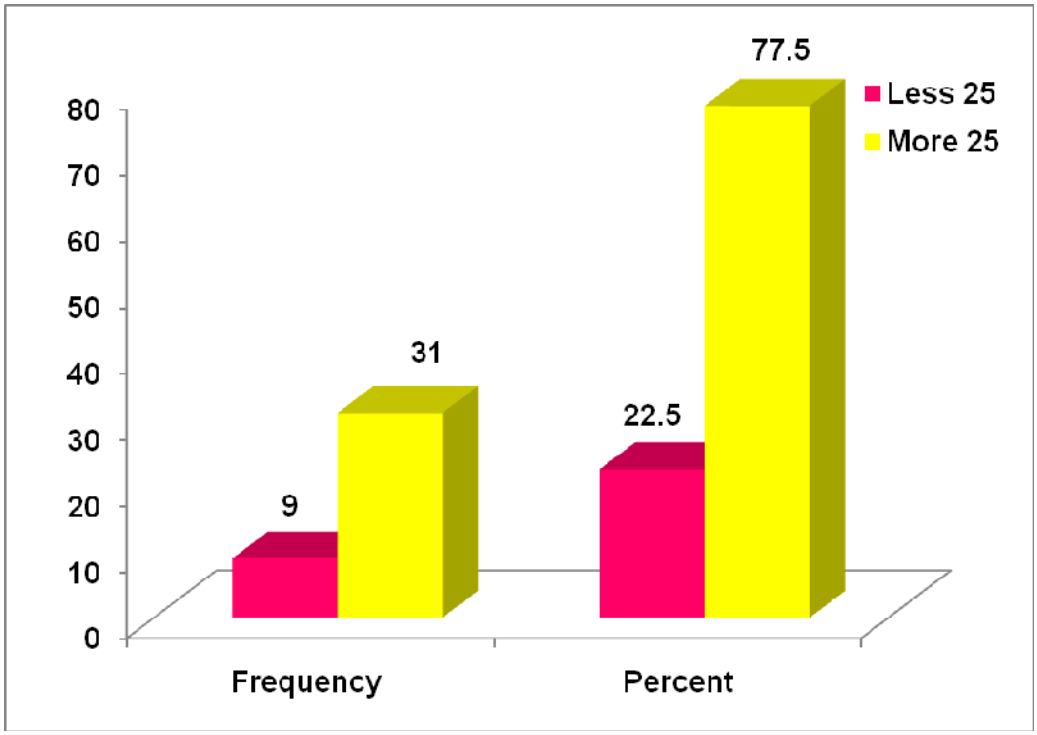
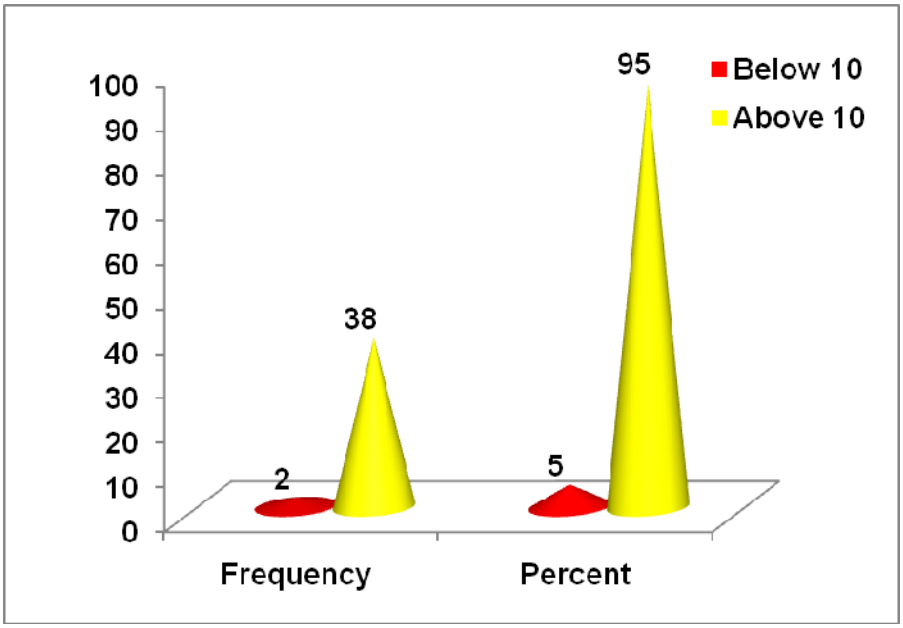


FIG – XII
FREQUENCY CHART FOR NEOPTERIN



FIG– XIII
FREQUENCY CHART FOR HISTORY OF
PREVIOUS HOSPITALIZATION

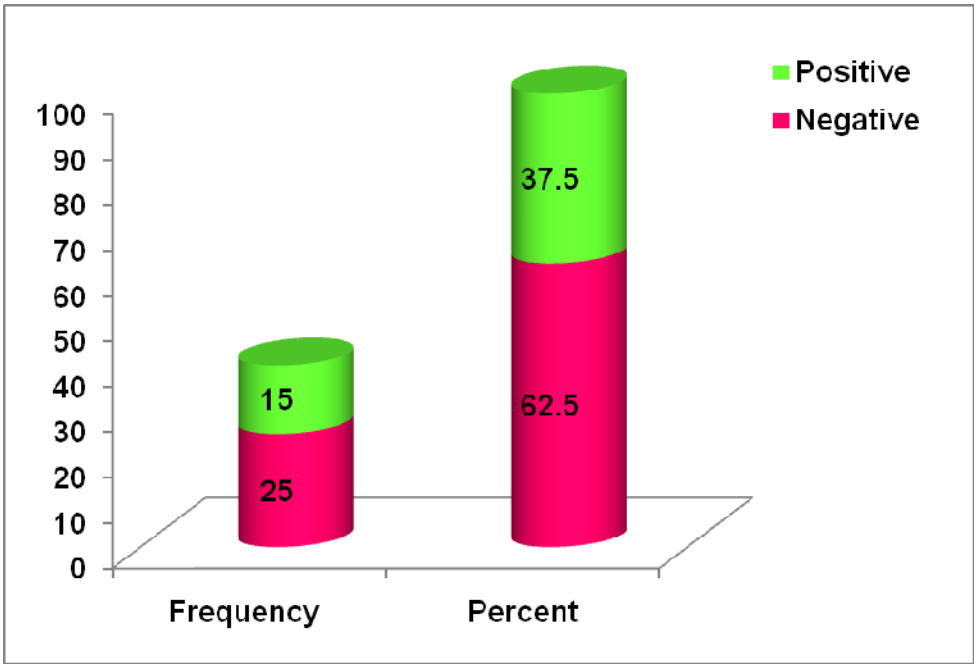


FIG – XIV
FREQUENCY CHART FOR MACE

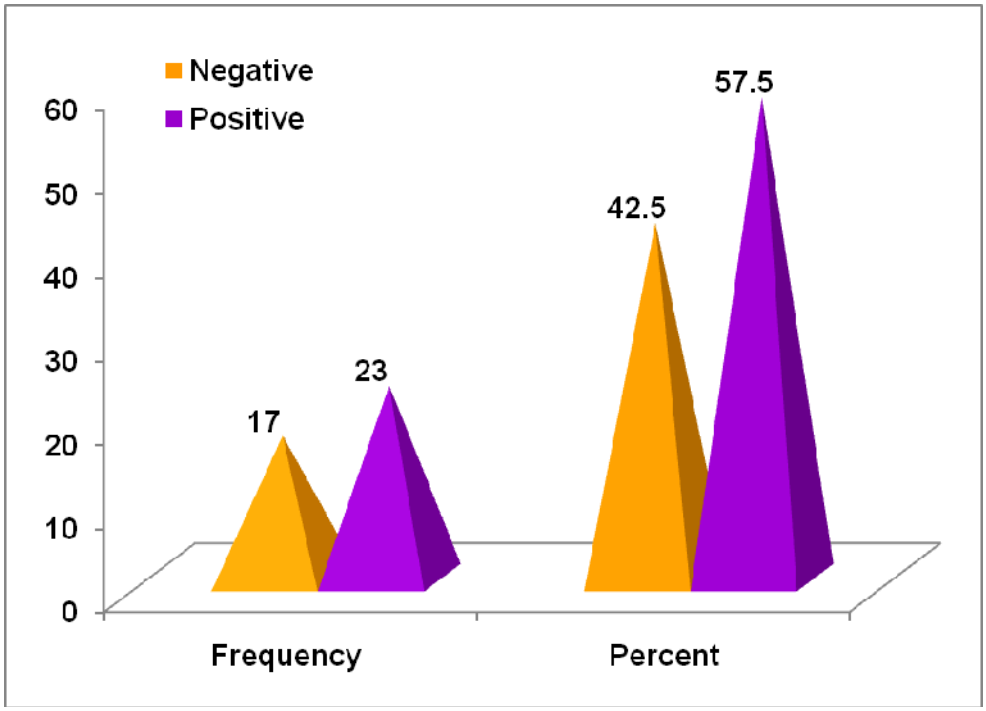


FIG – XV
FREQUENCY CHART FOR DEATH

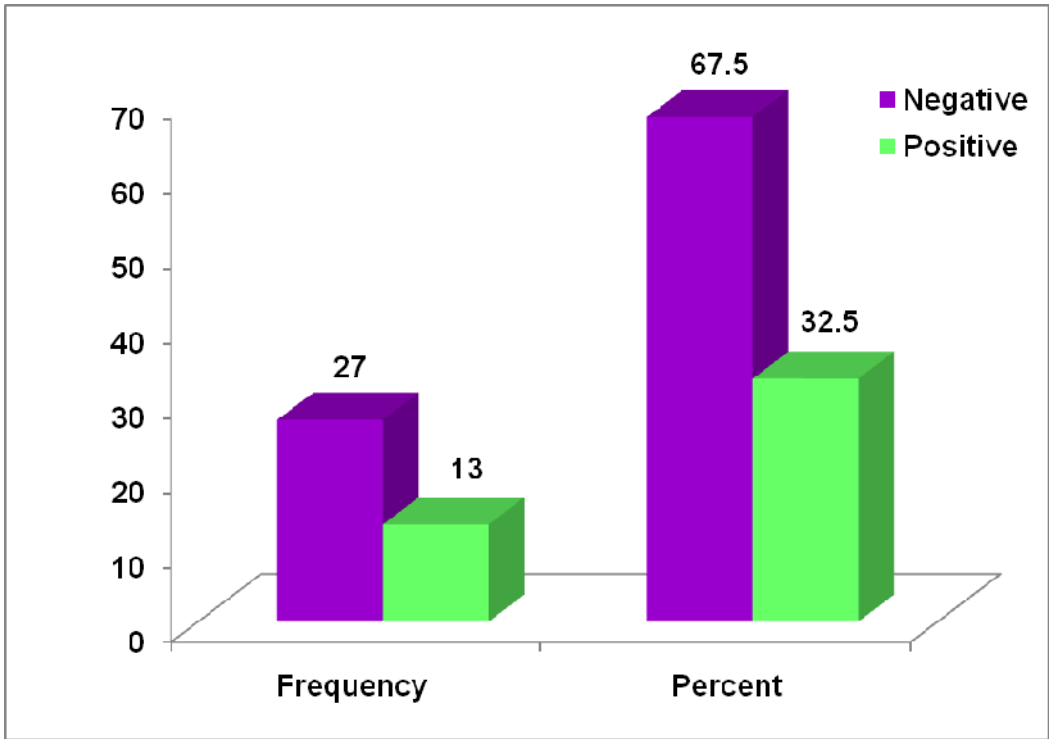


FIG – XVI

FREQUENCY CHART FOR SERUM CHOLESTEROL

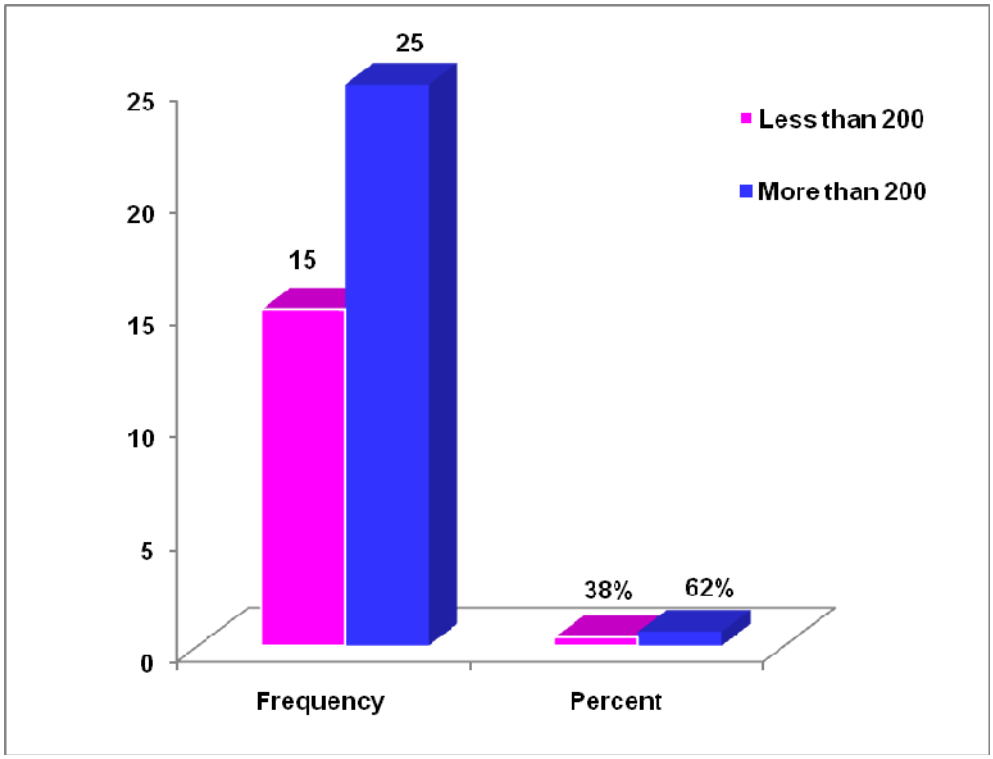
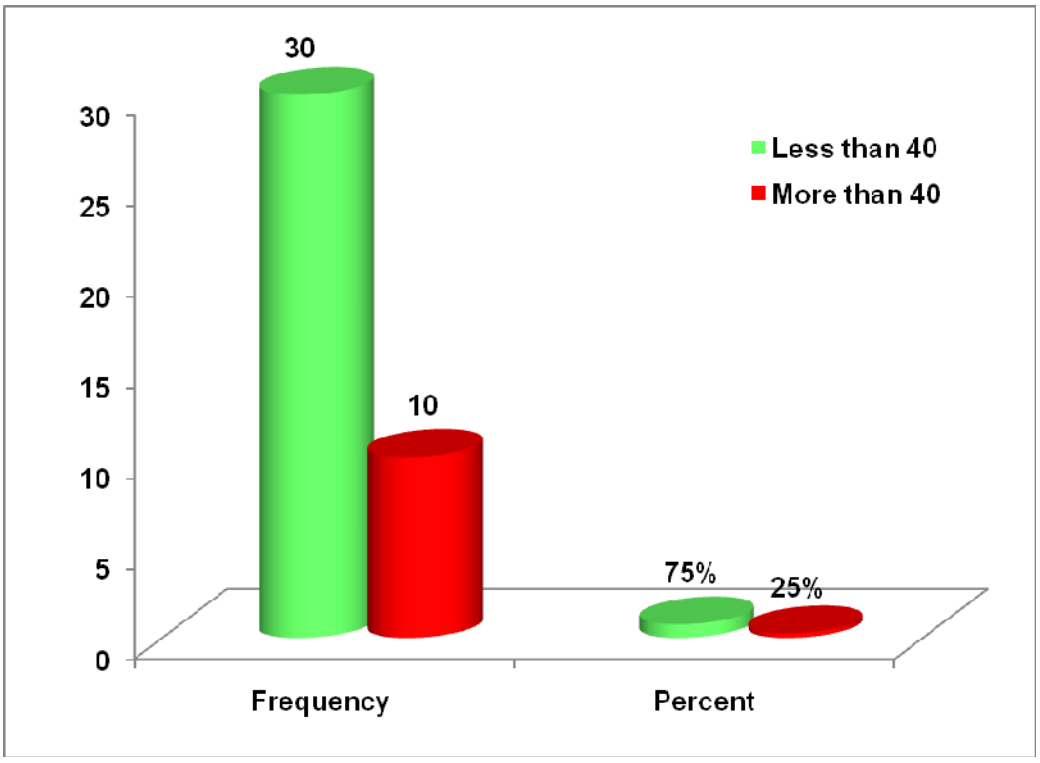


FIG – XVII
FREQUENCY CHART FOR SERUM HDL



MASTER CHART
TESTS

Sl. No.	Age	Sex	BP	FBG	CK-MB	N	PH	MACE	D	Sr.Choles	TGL	HDL	LDL	VLDL
1	52	I	120/80	120	42	99.6	IV	IV	IV	238	139	30	180	28
2	55	I	110/76	140	51	11.4	III	III	III	258	114	39	196	23
3	62	II	100/78	146	26	10.6	III	III	III	216	124	47	144	25
4	48	I	140/96	92	22	71.7	IV	IV	IV	192	106	34	137	21
5	45	I	130/92	86	56	6.9	III	III	III	213	132	46	141	26
6	56	II	160/94	106	82	26.9	IV	IV	III	144	137	26	91	27
7	58	I	170/98	100	26	29.5	III	IV	III	200	117	36	141	23
8	60	I	120/76	76	24	20.1	III	III	III	227	114	41	163	24
9	44	I	110/86	160	36	30.3	IV	IV	IV	189	119	22	143	24
10	59	II	100/70	76	30	12.2	III	III	III	222	126	33	164	25
11	50	I	160/100	110	62	26.9	IV	IV	IV	151	103	36	94	21
12	54	I	120/82	96	74	29.5	IV	IV	III	192	115	36	133	21
13	55	I	110/76	90	86	9.36	III	III	III	185	122	36	125	24
14	64	II	148/100	110	61	20.1	III	III	III	189	180	22	131	36
15	60	II	136/92	130	58	20.6	III	III	III	259	114	39	196	23
16	55	I	120/80	170	67	30	IV	IV	IV	202	104	52	129	21
17	60	II	110/72	86	88	18.3	III	III	III	216	124	47	144	25
18	42	I	170/94	112	44	30.5	IV	IV	IV	178	124	40	113	25
19	58	I	160/100	136	36	34.4	III	IV	IV	172	113	41	108	23
20	59	I	110/82	76	29	34.9	IV	IV	III	260	133	41	192	27
21	60	I	110/76	100	37	26.4	III	IV	III	166	134	22	117	27
22	65	II	120/80	90	40	13.2	III	III	III	276	176	20	221	35
23	50	I	142/98	160	27	80.9	IV	IV	IV	189	119	33	132	24
24	54	I	160/100	90	20	36.2	IV	IV	IV	194	134	37	130	27
25	56	I	110/82	84	46	17.1	III	III	III	227	136	22	178	27
26	51	II	110/72	90	62	28.9	III	IV	III	236	136	33	176	27
27	52	I	142/86	100	24	19.5	III	III	III	241	125	22	194	25
28	64	I	160/90	110	20	31.2	III	IV	IV	203	120	40	139	24
29	62	I	160/100	90	22	74.4	IV	IV	IV	201	165	33	135	33
30	56	I	120/80	130	34	21.5	III	IV	III	264	143	43	192	29
31	54	I	120/80	120	38	28.1	IV	IV	III	241	133	31	183	27
32	58	I	124/82	122	20	15.3	III	III	III	144	137	26	91	27
33	62	II	110/72	100	76	14.7	III	III	III	299	144	51	219	29
34	61	I	150/96	116	21	19.63	III	III	III	213	132	46	141	26
35	54	II	140/90	96	38	16.1	III	III	III	251	123	27	199	25
36	59	I	120/82	110	44	19.36	III	III	III	179	160	31	116	32
37	65	I	120/80	122	18	32.5	IV	IV	III	226	127	38	163	25
38	60	II	110/72	122	26	32.6	III	IV	IV	189	119	22	143	24
39	59	II	100/76	116	46	44.6	IV	IV	IV	177	134	30	120	27
40	62	I	120/80	100	82	26.2	III	IV	III	258	133	39	192	27

CONTROLS

41.	65	I	120/80	115	20	5.5	III	III	III	127	150	25	72	30
42.	55	I	120/80	120	16	6.2	III	III	III	158	133	39	92	27
43.	61	I	130/94	110	26	8.6	III	III	III	179	160	31	116	32
44.	58	II	120/80	92	25	7.6	III	III	III	176	170	40	102	34
45.	42	I	160/100	106	20	9.63	III	III	III	141	115	43	75	23
46.	64	I	120/80	86	88	5.6	III	III	III	171	122	40	107	24
47.	60	I	120/82	100	42	6.2	III	III	III	173	145	45	99	29
48.	59	I	160/100	96	40	9.2	III	III	III	156	130	32	98	26
49.	60	II	120/80	100	24	9.72	III	III	III	186	126	49	112	25
50.	52	I	120/80	102	25	7.8	III	III	III	171	122	40	107	24
51.	53	I	120/82	100	22	5.05	III	III	III	172	113	41	108	23
52.	56	I	120/80	90	20	7.09	III	III	III	145	120	43	78	24
53.	46	I	140/90	160	18	6.03	III	III	III	195	135	50	118	27
54.	50	I	112/82	100	15	7.8	III	III	III	165	145	49	87	29
55.	51	II	118/82	140	24	5.25	III	III	III	183	138	51	114	28
56.	48	II	150/96	126	19	6.05	III	III	III	148	152	48	69	31
57.	64	I	140/86	86	12	5.05	III	III	III	159	130	52	81	26
58.	63	II	122/82	72	23	9.82	III	III	III	179	142	50	119	28
59.	65	I	144/100	112	17	6.06	III	III	III	188	98	45	115	18
60.	55	I	120/80	96	26	4.62	III	III	III	193	100	43	130	20
61.	65	I	120/84	126	16	6.26	III	III	III	156	160	42	82	32
62.	62	II	126/82	82	11	5.92	III	III	III	150	86	45	46	18
63.	45	I	120/80	100	20	6.5	III	III	III	148	126	51	50	25
64.	51	I	140/96	100	22	7.01	III	III	III	145	72	59	72	14
65.	56	II	130/80	136	19	8.92	III	III	III	161	155	46	84	31
66.	60	II	122/82	172	24	6.62	III	III	III	186	138	52	106	28
67.	55	I	146/80	166	25	5.72	III	III	III	172	126	49	98	25
68.	48	I	120/80	76	11	6.06	III	III	III	160	140	66	66	28
69.	52	I	160/90	100	10	8.04	III	III	III	138	140	52	58	28
70.	45	I	120/82	96	12	6.06	III	III	III	152	126	60	67	25
71.	60	II	120/82	144	22	6.06	III	III	III	145	72	59	72	14
72.	62	II	110/80	100	20	7.02	III	III	III	150	86	35	97	18
73.	55	I	120/84	100	21	8.22	III	III	III	148	126	51	72	25
74.	50	I	120/80	112	15	6.45	III	III	III	167	141	47	92	28
75.	49	I	110/72	100	19	5.23	III	III	III	171	165	45	93	33
76.	55	I	120/84	100	21	8.22	III	III	III	148	126	51	72	25
77.	50	I	120/80	112	15	6.45	III	III	III	167	141	47	92	28
78.	49	I	110/72	100	19	5.23	III	III	III	171	165	45	93	33
79.	60	II	120/72	82	24	5.63	III	III	III	154	155	49	74	31
80.	52	I	110/80	76	20	4.66	III	III	III	200	128	58	120	22

I : Male ; II : Female ; III : Negative; IV : Positive

For BP – A & B
A = Systolic BP1
B = Diastolic BP2

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